Rapid solubility measurement with full automation using low sample weights

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Abstract

Purpose: pH-metric methods for measuring solubility of ionizable drugs provide results for kinetic and equilibrium solubility and supersaturation ratio in 1 hour experiments. However they require 10 – 30 mg of sample to run on the automated instruments currently available. The goal of this work was to measure solubility using no more than 1mg of sample, with all instrumental steps automated.

Methods: An instrument was devised (**Sirius T3**) using miniaturised components (pH electrode, overhead stirrer, temperature probe, capillaries for liquid additions and UV dip probe), together with robotic actions for probe and sample movement, enabling automatic titrations in 1mL solution volumes and automated clean-up between samples. This instrument was used to measure solubility of 10 drugs including diclofenac, imipramine and piroxicam, using samples of no more than 1mg, following published methods [1,2] that have previously been applied for larger samples.

Results: Equilibrium solubilities of 1.1µg/mL, 18.4µg/mL and 5.5µg/mL were measured using 1.0mg of diclofenac, 0.9mg of imipramine and 1.0mg of piroxicam. These results compare well with published values measured by the saturation shake-flask method. Kinetic solubilities were also measured, from which supersaturation ratios of 44, 1.0 and 39 were derived.

Conclusion: It has been shown that fully automated rapid pH-metric solubility measurements of ionizable drugs can be done using no more than 1mg of sample.

SiriusT3 (figures 1 & 2) 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 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, including a flowing water wash to clean 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.

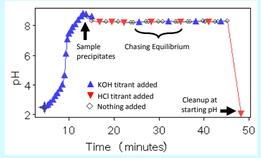
Figure 1 (above). Sirius T3 with Autoloader

Figure 2 (right). Sirius T3 single sample version

pH-metric solubility illustrated for bases

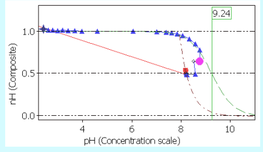
THE EXPERIMENT

This example is a **base** and a **chaser**. A weighed sample is dissolved at pH 2.5 (as BH^+), and then titrated with 0.5M KOH until it precipitates from a **supersaturated solution**. After the pH stabilizes, **Chasing Equilibrium** begins. Small volumes of HCl ▼or KOH ▲ titrants are added, and the rate of pH change is monitored. Adding HCl converts B to BH^+ and consumes H^+ ions; the sample dissolves, and the pH goes up. Adding KOH converts BH^+ to B and releases H^+ ions; the sample precipitates, and the pH goes down.



THE BJERRUM CURVE

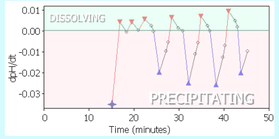
The titration curve is re-plotted as a Bjerrum curve. The points on the green dashed line show data up to the point of precipitation. The Y = 0.5 intercept denotes the aqueous pK_a. The pink circle denotes the precipitation point, which is used to determine the kinetic solubility. All points while chasing equilibrium fall on the Precipitation Bjerrum Curve (brown dashed line).



GRAPH OF $\frac{dpH}{dt}$ VERSUS TIME

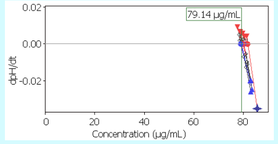
Just before the pH of each point is recorded, the rate of change of pH $\frac{dpH}{dt}$ is also recorded, and plotted vs. time. For bases*, rates are positive when samples are dissolving, and negative when they are precipitating. At the crossing points when rates are zero, the sample is at equilibrium solubility.

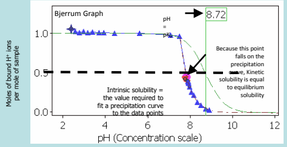
** opposite for acids*



THE EQUILIBRIUM CHASING CROSSOVER PLOT

Using mass balance and charge balance equations, the concentration of B in solution is calculated at each point. When consecutive points are plotted, they intersect on the Y = 0 axis at a concentration equal to the **equilibrium solubility**. The solubility of this sample is 79.14µg/mL.





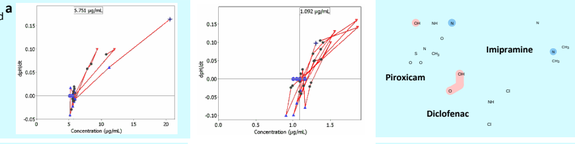
This sample is a **base** and a **non-chaser**. The neutral species does not form a supersaturated solution. It precipitates as soon as the concentration of neutral species in solution is equal to the intrinsic solubility. The precipitation point, as well as all pH points after precipitation fall on the precipitation Bjerrum curve. The kinetic solubility of non-chasers is equal to their intrinsic solubility. Solubility is determined by fitting the data to the theoretical Bjerrum Curve. The solubility of this sample is 48.7µg/mL.

Solubility on Sirius T3 using 1mg sample weights

a. Equilibrium Chasing Crossover Plot. Measured solubility = the mean intercept value.

b. Bjerrum curves. Green curve represents the aqueous pK_a values (required for assay). Brown curve is the precipitation Bjerrum curve, calculated from fitting mass and charge balance equations to the experimental data.

c. Assay settings from **Sirius T3** software, showing the weights of sample. All assays were done in 1mL total solution volumes



Compound	Sirius T3 µg/mL	Literature µg/mL
Chlorpromazine	2.1 2.2 2.3	2.7
Piroxicam	5.5 5.7 5.3 5.9	5.9
Imipramine	18.4 15.4	17.3
Niflumic acid	9.0 9.9	9.6
Diclofenac	1.1 1.1 1.1	1.1
Verapamil	53.3 52.7	48.7
Warfarin	6.4 5.3	5.2
Amitriptyline	8.8 7.3	11.3
Glipizide	1.3 1.6	1.4
Promethazine	16.5 16.6	18.4

Table 1. Intrinsic solubilities measured on Sirius T3 using ~1mg of sample per assay, vs. published pH-metric values using 10-20 mg of sample [3].

Solubility of Piroxicam

1.05mg of piroxicam was weighed into a sample vial. 1mL of aqueous 0.15M KCl was added and the pH was raised to 11.5 with 0.5M KOH. The sample was then titrated with 0.5M HCl until it precipitated at the Kinetic point. Precipitation was detected by a UV turbidity probe.

The sample chased equilibrium over the pH range where precipitate was present, aided by the additions of small aliquots of KOH and HCl.

Note that the pK_a of a moderately soluble sample can be determined from a solubility experiment, by fitting the green curve to the data collected before precipitation. Thus pK_a and solubility can be obtained from the same experiment.

Solubility of Diclofenac

0.94mg of diclofenac was weighed into a sample vial, and analysed following a similar method to that used for piroxicam.

The solubility result 1.092µg/mL is shown in the Equilibrium Chasing Crossover Plot.

Diclofenac and piroxicam form supersaturated solutions and first precipitate at the kinetic point in a relatively soluble form which is probably amorphous. They subsequently convert to a crystalline form with lower Intrinsic solubility which is represented by the CheqPoint.

Solubility of Imipramine

1.2mg of imipramine was weighed into a sample vial. 1mL of aqueous 0.15M KCl was added and the pH was lowered to 2.5 with 0.5M HCl. The sample was then titrated with 0.5M KOH until it precipitated at the Kinetic point. Precipitation was detected by a UV turbidity probe.

Imipramine does not form a supersaturated solution under the experimental conditions, and remains in the initial precipitated form throughout the experiment.

A recent publication [3] provides in-depth discussion about supersaturation and its effects on drug physicochemical properties.

Conclusions A new instrument (**Sirius T3**) and pH-metric methodology has been described for measuring solubility of drugs using 1mg sample weights. Results for 10 drugs compare well with values measured by pH-metric methods using older instrumentation and larger sample weights.

References

[1] Stuart, M. Box, K. Anal. Chem. 2005, 77(4), 983-990

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