Aqueous Solubility Measurement – Kinetic vs. Thermodynamic Methods

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Introduction

Turbidity-based solubility determinations, spurred principally by the highly publicized work of Pfizer's Chris Lipinski, have gained popularity in many drug discovery companies. The turbidity based method, also known as "kinetic solubility," is often compared to the tried and true method of "thermodynamic solubility." Thermodynamic often conjures images of shake flasks and laborious experiments, but new technology for both methods are gaining a foothold in the industry.

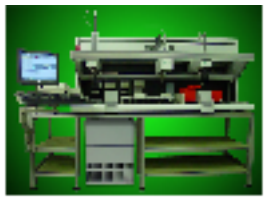
The key difference between the two terms is related to *time*. Kinetics is a time-dependent phenomenon, and thermodynamics is, by definition, time independent. Other issues, such as crystal sample vs. amorphous-sample/DMSO-stock solution sample are not as significant differentiating factors as time. For example, it is possible to measure thermodynamic solubility of a compound in the presence of any amount of DMSO, but is is highly improbable to measure this over a very short period of time (< 10 minutes).

Usually, solubility is over-estimated by the "kinetic" method, compared to the "thermodynamic" method. That is, the actual solubility can be much less than the former method determines, especially for very insoluble test compounds. This could result in problems downstream, when unanticipated precipitation takes place at the site of injection in rats (often killing the *in vivo* assay), as part of lead optimization projects.

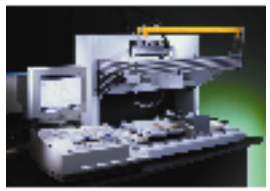
As one watches what happens when a small aliquot of a 10 mM DMSO stock solution of a sparingly-soluble compound is added to an aqueous buffer in a test tube, one often sees the almost immediate formation of an opaque or hazy, turbid suspension. It can have a grayish or bluish tinge to it, depending on compound. After about ten minutes, the suspension seems to become less opaque. After about one hour, in many cases, the solution seems to "clear up" mysteriously. But on close inspection with a magnifying glass and a bright flashlight, one sees micro-crystals formed, non-uniformly distributed: usually attached to some rough surface, or just floating on the surface of the solution, sometimes crawling up the meniscus. Suspensions of extremely insoluble compounds, such as terfenadine or amiodarone, may remain turbid for days or weeks.

Purpose

This poster compares the results of thermodynamic vs. kinetic assays performed using *p*ION's patented Modified Shake Flask Method (the "µSOL" method). Results for the same compounds, but run through different protocols will be analyzed and compared. This should help chemists better understand which protocol may be best for use in their lab.



The µSOL Discovery is a very high-throughput platform for solubility analysis. Discovery performs only kinetic solubility assays in a 384 well plate format. It is capable of processing well over 1,500 assays per day.



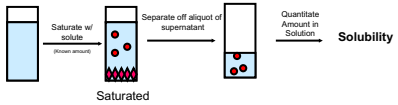
The µSOL Evolution is a high-throughput platform for solubility analysis. Evolution is available in both kinetic and thermodynamic modes. It is capable of processing 400-600 assays per day.

The µSOL Explorer can be used manually or can be easily added to your existing liquid handling system. Explorer provides users with a license to the *p*ION solubility method and our proprietary software. Explorer can perform both kinetic and thermodynamic assays.

How It Works

The µSOL solubility analyzer makes use of a patented modified shake-flask method shown below. Samples are introduced from DMSO stock, then, depending upon the protocol, are immediately processed (kinetic) or incubated for at least 12 hours (thermodynamic). Aliquots of supernant are removed and analyzed for sample concentration. If material is precipitated (and thus "lost" from solution) after incubation then the solubility can be determined. If material is not lost then solubility is equal to or higher than the concentration assayed.

The general method:



Samples are normally introduced as 10 mM DMSO stock solutions from 96 well plates. For speed of analysis, UV detection is used. A 96 well plate can be read in only 15 minutes! In our method we use proprietary data analysis methods to allow the use of only a single reference sample. Concentrations of compounds without UV detection require the use of LC-MS methods which can be easily imported into our software. Samples never have more than 1% DMSO.

When using the direct UV plate methods to determine solubility, such as the *p*ION µSOL Evolution (robotic) or Explorer (manual, low-cost) method, the turbid or crystal-containing solution is filtered (or centrifuged), and the concentration of the dissolved species is measured by spectroscopy, from which solubility is derived.

Many discovery scientists don't realize that "kinetic solubility" can be determined by this UV method. But it can! The key distinction between the two types of solubility is *time*. If the filtration/ centrifugation is done in the first 1-10 minutes, one gets *kinetic* results by UV. If the solution is allowed to first sit overnight, and then is filtered/centrifuged, one gets *thermodynamic* results by UV. It's as simple as that.

The µSOL method is different from all other UV plate-based methods. It is "self-calibrating." NO DILUTION CURVE IS NEEDED, which dramatically simplifies and *speeds* up the assay.

COMPOUND	CAS No.	FW	Type	pH	S average T, µg/mL	S average K, µg/mL
Diclofenac.Na	15307-79-6	318.1	Acid	3	0.8	44
Progesterone	57-83-0	314.5	Neutral	3-10	23	61
Griseofulvin	126-07-8	352.8	Neutral	3-10	30	24
Chlorpromazine. HCl	69-09-0	355.3	Base	10	1.5	1.2
Flurbiprofen	5104-49-4	244.3	Acid	3	21	44.9
Indomethacin	53-86-1	357.8	Acid	3	0.9	33
Phenazopyridine. HCl	136-40-3	249.7	Base	7.4	24	24

Table. Examples of kinetic and thermodynamic solubility results obtained by µSOL Evolution instrument. Sample solutions contained 1% DMSO in both cases.

Summary

Thermodynamic numbers can be different from kinetic numbers, since it takes time for crystals to form from the initially precipitated amorphous material. It takes time for crystals to become large enough not to pass through the microfilter and be treated as "dissolved."

The transit time through the intestine is only 2-4 hours. If precipitation takes place at the start of the descent through the intestine, nice crystalline material may not have enough time to form. It may be proposed that "kinetic" solubility is the relevant number to use under clinically-relevant conditions.

As can be seen from the table above, some compounds are very sensitive to how long the saturated solution stands, and some compounds are not, in terms of the solubility observed.

In lead optimization, when animal measurements are done, it may not be possible to predict this phenomenon, and it may be easier just to measure the solubility under *in vivo*-relevant conditions, that is, as "kinetic".

In preformulation, "thermodynamic" solubility is needed to predict the rates of dissolution of development compounds.