INTRODUCTION

Solubility is an important parameter for the development of drugs, from early discovery and lead optimisation where it helps with the identification of potential bioavailability issues, to drug development and formulation where solubility knowledge is required for biopharmaceutical classification, biowaivers and bioequivalence. Also, good, reliable and reproducible solubility values will improve the in silico approaches for predicting solubility from structure.

Knowledge of solubility is a challenge for the drug industry because the measured values are dependant on the experimental conditions, and because different solubility concepts are not always mentioned when comparing the results. Although there is a lack of solubility standardisation, a few definitions can be placed at the core of solubility measurements. **Equilibrium solubility** is the concentration of a compound in a saturated solution when excess solid is present and the solution and solid are at equilibrium. The gold standard method for the measurement of equilibrium solubility is the classical shake-flask method, accurate but slow. **Intrinsic solubility** is the equilibrium solubility of the neutral form of the molecule. **Kinetic solubility** is defined as the compound’s concentration measured immediately after the induced precipitate first appears in the solution. Many methods are used for kinetic solubility screening of large numbers of compounds, while the selected candidates will be re-measured for more accurate solubility profiles. A typical throughput for solubility screening is 50-100 samples/day and minimum sample consumption is desirable. Several methods are used for solubility screening: turbidity, nephelometry, cytometry, direct spectrometry etc. The sample requirements can be maintained at a minimum value by using stock solutions in organic solvents (DMSO is the most used solvent) in 96-well plates (1). The kinetic approach has the advantage of providing a rank order for solubility which can be then accurately measured in later stages of drug development. Kinetic solubility is quick to measure, but the result is often much higher than the equilibrium solubility.

Getting the solubility right presents many challenges, including experimental design, data interpretation and inter-laboratory comparison. This paper describes an innovative, patented approach to measure both kinetic and intrinsic solubility, as well as providing a full solubility pH profile, from only one experiment. CheqSol (Chasing
Equilibrium Solubility) involves a modified pH-metric approach applied to small ionisable organic molecules (2,3).

**WHAT IS CHEQSOl?**

CheqSol is a modified pH-metric method where a fully ionised, dissolved compound is brought under precipitation conditions by repeatedly adding small amounts of acid or base, depending on the pH range where the sample is fully dissociated. For example an acid will be fully dissociated at high pH values and will have to be titrated with an acid titrant to lower the pH and seek precipitation, while a base will be fully dissociated at low pH values and will be titrated with a base towards precipitation. The amount of sample must be accurately measured then dissolved at a suitable pH in an aqueous solution with the ionic strength adjusted to 0.15M with KCl. This ionic strength is similar to blood ionic strength and helps simulate in vivo conditions.

The next step involves the titration of the ionised compound in solution by adding measured aliquots of acid or base until the solution becomes cloudy, signalling the beginning of precipitation. The occurrence of the precipitation can be detected by just observing the experiment, or by using a UV dip probe. This is the moment when kinetic solubility can be measured.

After this stage, the experiment is forced to “chase equilibrium” by repeatedly adding small amounts of acid or base titrant to direct the sample towards precipitation or re-dissolution (2,3). The example in Figure 2 shows the data collected during a CheqSol experiment on Diclofenac.

**Diclofenac** is a potent nonsteroidal anti-inflammatory drug. Diclofenac can be considered a monoprotic acid in the measurable pKa range of 1-13 (Figure 1), with the measured pKa = 3.99 at 25ºC and 0.15M KCl ionic strength. Diclofenac is fully dissociated in a solution at pH 12. The anionic Diclofenac has to extract a charged H⁺ (or H3O⁺) to form the neutral molecule and precipitate from the solution. This process reduces the H⁺ concentration in the solution and a sensitive instrument will be able to monitor a rise in pH during the precipitation process.

In a CheqSol experiment, the titrator (GLpKa, Sirius Analytical, UK) will add measured aliquots of acid and the base to the Diclofenac solution, “forcing” Diclofenac to precipitate or re-dissolve depending on the titrated added (acid or base). If the amount of added titrants is not sufficient to complete the precipitation or dissolution process, they will just start these processes. Monitoring the pH changes in the solution, when no titrant is added, shows a positive pH gradient when Diclofenac precipitates and a negative pH gradient when it re-dissolves. The pH gradients are a consequence of the H⁺ displacement from the solution to the neutral form of the molecule and back. This gradient is very small, sometimes only the second and third decimals of the pH values change, and can be detected only by sensitive instrumentation.

Figure 2 shows a plot of the pH gradient vs. time for the experiment described for Diclofenac. The experiment starts at pH 12 where Diclofenac is fully dissociated. This point is marked on the graph with a blue star. Diclofenac starts precipitating at pH 4.96. The blue lines ending with blue triangles show the pH gradient after base titrant was added to the solution, the red lines ending with red triangles show the pH gradient after acid titrant was added, and the black lines ending with black circles show the pH monitored during precipitation or re-dissolution, when no titrant was added. Figure 2 shows eight points crossing the horizontal line where the pH gradient is 0 and where the system will be at equilibrium. The intrinsic (equilibrium) solubility is calculated as the average value of the eight concentrations at the eight crossing points. Diclofenac’s measured intrinsic solubility is 1.015 µg/mL.

**How is Solubility Measured?**

A carefully designed CheqSol experiment will take into consideration the accurately measured weight of sample, information about the structure of the sample and if the sample is a salt, the total volume of solution, the standardised concentrations and volumes of acid and base dispensed, pH readings at each point on the titration/precipitation curve, the charge of counterions (if the sample is a salt), and accurate pKa values for the sample including the type (acid or base). All this information allows the continuous calculation of the distribution of species using the equations corresponding to the acid-base equilibrium and mass balance, knowing the distribution of species means that the amount of dissociated sample, e.g. present in solution, can be calculated at every point during the experiment. Accurate pKa values are required for reliable solubility values because any error affecting the pKa will
be propagated on a logarithmic scale onto the solubility value.

**The Theoretical Titration and Precipitation Curves**

Titration curves of weak acids and bases can be plotted as Bjerrum curves, showing the concentration of dissociable protons (nH) vs. pH. Figure 3 shows the theoretical aqueous (green) and precipitation (purple) Bjerrum curves for Diclofenac. The aqueous curve can be obtained when Diclofenac’s concentration is below the value required for precipitation and the solution is titrated with HCl. The number of exchanged H⁺ ions per molecule (nH) is represented on the Y scale, which for Diclofenac ranges from 0 to 1. The pH when nH = 0.5 is called the pKa. At this pH, 50% of the Diclofenac molecules are ionised, while the other 50% are neutral. The precipitation curve could be obtained with a classical pH-metric titration on a more concentrated solution, when Diclofenac precipitates if the right experimental conditions are met.

In a traditional shake-flask experiment, the sample is left to completely precipitate by waiting at least 24 hours to make sure that the precipitation process is finalised and the equilibrium is achieved. The sample concentration in the supernatant is then measured by a suitable method, usually spectrometry, ensuring that the solution is separated from the precipitate.

CheqSol cuts this waiting time by “chasing equilibrium” around the equilibrium solubility value, and calculates it from the amount of dissociated sample, instead of waiting for equilibrium. In Figure 3, the start of the CheqSol experiment is marked by the blue star after which red triangles denote the readings while Diclofenac ions are in solution, until Diclofenac starts precipitating (point marked by the purple circle, from which kinetic solubility is derived) and moves to chase equilibrium around a point situated on the theoretical precipitation curve. It is obvious from the graph that in this case, the kinetic solubility is different from the intrinsic solubility. The kinetic solubility for Diclofenac is 43.6 µg/mL, 44 times higher than its intrinsic solubility.

A validation study comparing the intrinsic solubility values obtained with CheqSol and with the standard shake-flask method has been published (4).

Carbon dioxide from air can have unpredictable effects on CheqSol experiments because it dissolves in water as carbonic acid, which can also be titrated and has two measurable pKas. Moreover, insoluble carbonate salts are sometimes formed in addition to the sample precipitate. As a precaution, all experiments should be done under a blanket of inert gas, preferably argon, but nitrogen can also be used.

**Cosolvent Assays**

Cosolvent assays can be used when the ionised form of the sample doesn’t dissolve in aqueous solutions. Any cosolvent can be used as long as it is fully miscible with water, has relatively low volatility and the electrode is calibrated for this system. Usually the solubility is measured in at least three different cosolvent concentrations, then extrapolated to pure water (Figure 4). Validation studies on samples that can be measured in aqueous solution show that the extrapolation is linear in water-rich solutions.

The solubility value, logS, measured for Diclofenac directly in an aqueous solution was -5.55. Figure 4 shows the extrapolated aqueous solubility value from five experiments at different methanol concentrations, which is -5.49.

**Information Provided by “Chasing Equilibrium”**

Chasing equilibrium is a property that not all ionisable compounds have. It is directly linked with the capacity of the compound to form supersaturated solutions. If a compound supersaturates, it will have a kinetic solubility much higher than its intrinsic solubility. The kinetic solubility values obtained from screening procedures have to be used carefully, as it is the intrinsic solubility the parameter that defines the solubility-pH profile of a compound.

“Chasing equilibrium” will provide kinetic and intrinsic solubility values from only one experiment, faster than other methods, and also provides a richness of information useful for other applications. Some of this additional information is discussed below.

**Precipitation and Dissolution Rates - The Four-Class Model**

The measured positive and negative pH gradients provide information about the precipitation and dissolution rates in the region of equilibrium. If these rates have similar values, the “chasing equilibrium” plot shown in Figure 2 will be symmetrical. If one of these rates is significantly higher that the other one, the graph will look asymmetrical.

Many compounds show a tight symmetry (diclofenac, piroxicam, sulfamerazine). Other compounds show a clear offset, indicating that the precipitation rate is higher than the dissolution rate, or vice versa. Taking into consideration this behaviour, four classes of compounds can be defined according to their relative rate of precipitation and dissolution (Table I). Many compounds are “Chasers”, well behaved compounds that will chase equilibrium, producing nice, symmetrical plots. Another class, the “Non-chasers” don’t supersaturate and will not chase equilibrium during a normal CheqSol experiment. They follow the theoretical titration curve and precipitate immediately after they exceed

### Table I – The Four-class CheqSol model

<table>
<thead>
<tr>
<th>Slow Dissolver</th>
<th>Fast Dissolver</th>
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<tbody>
<tr>
<td><strong>“Chasers”</strong></td>
<td><strong>“Non-Chasers”</strong></td>
</tr>
<tr>
<td>Full chaser for both precipitation and dissolution</td>
<td>No precipitation chase but can chase while dissolving</td>
</tr>
<tr>
<td>Examples: Ibuprofen, Benzoic acid, Benzthiazide</td>
<td>Examples: Nortriptyline, Amitriptyline, Imipramine</td>
</tr>
<tr>
<td><strong>“Super Chasers” (“Super Dissolvers”)</strong></td>
<td><strong>“Ghosts”</strong></td>
</tr>
<tr>
<td>Chases during precipitation but not while dissolving</td>
<td>No chasing possible for either precipitation or dissolution</td>
</tr>
<tr>
<td>Examples: Tolmetin, Papaverine, Chloroxazone</td>
<td>Examples: None yet discovered</td>
</tr>
</tbody>
</table>
B. different polymorph, a chaser
A. non-chaser behaviour;

Figure 5 – Sulindac polymorph conversion. 
A. non-chaser behaviour; 
B. different polymorph, a chaser

the solubility limit. Their kinetic solubility always equals the intrinsic solubility. The Super-chasers (super-dissolvers) supersaturate and dissolve very quickly. The existence of the fourth class, the “ghosts” is only postulated because no compound that can precipitate fast and dissolve fast has yet been observed.

How Many Cycles Are Possible?
A stable, non-degradable compound, which precipitates as the most thermodynamically stable polymorph, will “chase equilibrium” for as long as the solution is sufficiently concentrated for the sample to precipitate. Studies on Warfarin and Sulfamerazine show up to 400 crossing points without a significant degradation of the cycling mode.

Measuring Very Low Solubility
CheqSol’s normal dynamic range is from mg/mL to μg/mL, but in exceptional cases it can measure aqueous solubility in the ng/mL or pg/mL ranges without any cosolvent. Two examples are Pamoic Acid and Amodiaquine. Both compounds have two close pKa values: Pamoic Acid has acid pKas of 2.65 and 3.35, while Amodiaquine has basic pKas of 7.37 and 8.24. Pamoic Acid and Amodiaquine are special cases because although the solubility of the neutral species is very low, the salts are soluble because the pKas are close together, and the ionic charge is +2 and -2, respectively. The measured aqueous intrinsic solubility for Pamoic Acid was 300 pg/mL, while for Amodiaquine was 400 ng/mL. In contrast, poorly soluble molecules with one pKa must be measured in water-solvent (e.g. miconazole) if the salt is insoluble.

Solubility at 37°C
The large majority of solubility experiments performed in pharmaceutical research and development are done at room temperature at 21-25°C. However, the drugs will be used at body temperature, at 37°C. Is this important? It could be. The solubility experiments using CheqSol showed that Diclofenac is 200% more soluble at body temperature that at the room temperature, Sulindac behaves as a non-chaser with a measured intrinsic solubility of 71.8 μg/mL (Figure 5A). After about 20 minutes, it starts to turn into a chaser with intrinsic solubility of 10.5 μg/mL (Figure 5B). This behaviour is repeatable for all CheqSol assays of Sulindac. The conversion between the two polymorphic forms was confirmed by Raman spectroscopy during the assay. Subsequent XRD was able to reveal a new crystalline form.

CONCLUSION
Solubility measurements are of crucial importance for the pharmaceutical industry. While rapid screening procedures allow the selection of drug candidates, more accurate measurements and detailed profiling is necessary in drug development. Comparing solubility data reported in the literature continues to be a challenge. Chasing equilibrium solubility is a reliable method that can be used to standardise the comparison of solubility data obtained in different laboratories. Measurements of solubility reported in the literature can differ significantly as it is hard to ensure that thermodynamic conditions have been reached. To test the CheqSol robustness, several assays were designed to start from different crystalline forms of the free form of Diclofenac and the sodium salt, repeated 10 times for statistical treatment (6). As expected the intrinsic solubility was the same for all forms because it refers to the free acid.

REFERENCES
3) www.cheqsol.com
4) Box K., Volk G., Baka E., Stuart M., Takaracs-Novak K., Comer J. J. Pharm. Sci. 2006, 96 (6), 1293-307

PHARMACEUTICALS