A measured solution

Researchers are using different techniques to address drug solubility issues.

BY JEFFREY RUELL AND ALEX AVDEEF

The tremendous investment in drug discovery since the early 1990s has resulted in far fewer drugs than expected. Instead, a plethora of high-affinity ligands has been produced. These compounds differ from drugs because they lack the pharmacokinetic and physicochemical properties necessary to be effective in vivo despite their remarkable in vitro biological activity. Facing this dilemma, industry has started to look at druglike properties early in discovery to sort through these high-affinity ligands. This approach has required the development of assays and screens that accurately measure key properties, such as permeability, pK_a, metabolism, and solubility (*1*).

Solubility is defined as the amount of material that remains in solution in a given volume of solvent containing undissolved material. It is a thermodynamic property of a compound, and the solubility of all drugs that go to market is well characterized. Until recently, however, this was not the case for compounds in the discovery phase. Many people mistakenly neglected this property, but upon reexamining the discovery process, the importance of knowing a compound's solubility becomes obvious. For example, biological assays require that compounds dissolve into the assay medium, and it might be necessary to verify this behavior experimentally before accepting an assay result. It might also be useful to identify false positives when solubility assays indicate that oligomers or aggregates are present that can act nonspecifically.

For the hit-to-lead decision process, solubility information can foreshadow future problems with a compound. Eventually, these compounds are going to be used in expensive animal tests that require that they not precipitate in plasma, producing toxic side effects. Identifying poorly soluble compounds can help alleviate this problem. And finally, a goal of most discovery work is to make compounds that are effective when taken orally. Therefore, it would be beneficial to identify compounds that will eventually need formulation for gastrointestinal absorption.

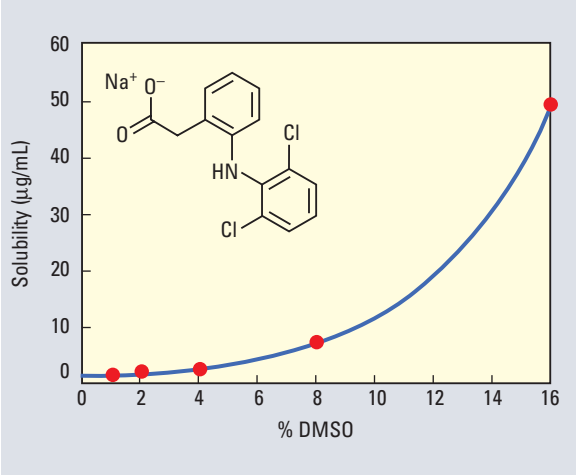

Figure 1. Solvent effects on solubility. The effect of DMSO on the solubility of diclofenac (structure) at pH 1.0 was measured on the µSOL solubility analyzer. (Figure courtesy of *p*ION Inc.)

The challenges

Predictive methods are normally used first to solve a problem. However, little standardization exists in the solubility data literature, and what is commonly known is limited to commercial drugs and not the huge numbers of structurally unrelated compounds produced today. This makes fragment analysis difficult, and the only researchers who can examine predictive studies are those few who have access to large proprietary databases. A few successful attempts have been made, but it is generally agreed that often a compound's partition coefficient (log P) alone is an indicator of solubility problems (*2*). As a result, solubility has become one of the few properties that experimenters have chosen to measure rather than model.

Despite their importance, solubility assays have only recently been introduced into the compound-profiling regimen. In fact, it has only been with the instrumentation explosion of the 1990s that the tools necessary to perform these assays, specifically the 96-well format and robotics, have become available. Developing these assays also required rethinking how to measure solubility. Because compounds are often maintained as dimethyl sulfoxide (DMSO) solutions and not as pure solids, the traditional solid precipitation method using the "shake-flask" technique is insufficient.

Solubility assays normally assume that a stock solution is homogeneous, but this is often not the case. Impurities from chemical synthesis might be present, which can complicate data interpretation, and the situation only gets worse when stock purity limits are decreased in favor of reduced costs. Also, given that the compounds are stored in DMSO, experimenters need to be aware of this solvent's effect on solubility. This point is illustrated in Figure 1 with diclofenac, a nonsteroidal anti-inflammatory drug. There is a clear exponential relationship between the amount of material that is soluble at pH 1.0, which mimics the stomach pH, and the amount of DMSO in the assay.

Although the effect is compound-specific, all compounds reach a point at which DMSO will grossly enhance solubility. The DMSO also limits the volume of sample that

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FIGURES: FIGURES ARE COURTESY OF THE AUTHORS.

can be added to an assay and therefore the amount of material that can be used. For a typical discovery-level solubility assay using 0.5–1 mL of buffer, only 10–50 µL of DMSO stock can be added before the results are completely erroneous. The exact amount of DMSO added, however, varies from company to company.

The DMSO effect can therefore limit the scope of solution precipitation assays. The assays are normally effective for observing solubility problems when compounds are present at very low concentrations (<0.5 mg/mL). They cannot be used to determine salt solubility accurately. The practical chemist, however, has learned to overcome these issues. Solution precipitation assays are particularly well suited for determining the intrinsic solubility of a neutral species (Figure 2).

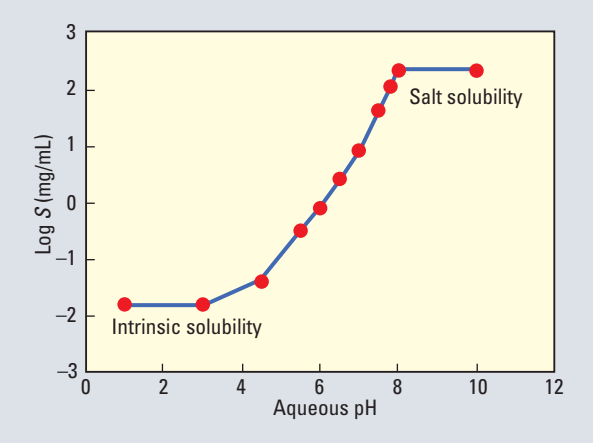

Figure 2. Intrinsic versus salt solubility. Intrinsic solubility is identified when the compound is uncharged (e.g., pH < 3) and is often within the dynamic range of solution precipitation methods. The salt solubility, however, is found at a much higher concentration (10,000 × intrinsic) and cannot be accurately measured or predicted. Results shown are those for naproxen sodium. (Figure courtesy of *p*ION Inc.)

Faced with working with existing sample formats and conditions, two solubility determination methods have become dominant—the particle detection and concentration-based methods. Particle detection uses physical measurements to determine the dilution at which precipitation stops, whereas concentration-based measurements focus on determining how much of a compound is in solution. The two approaches are vastly different, and their results have different applications.

Particle detection assays

Without question, one of the pioneers in developing and implementing solubility assays has been Pfizer's Christopher Lipinski. His work illustrates that not only can the solubility of discovery compounds be easily measured, but the problem of compounds with low water solubility is greater than one would think (*3*). He used turbidometric analysis, which was originally developed almost half a century ago to examine drinking water. This method determines where, on a dilution curve, turbidity (light absorption or scattering by a sample) no longer exists.

Turbidometric measurements are most useful to characterize kinetic solubility, when equilibrium between solid and solution phases may not be established. Kinetic solubility often does not correlate with equilibrium solubility measurements because the latter depend on crystalline polymorphic properties and crystal lattice energy. Thus, the turbidometric technique has been relegated to supporting high-throughput screening assays and quick yes–no answers concerning solubility at specific concentrations. As compounds go further into development, the turbidometric method becomes less reliable, and other measurements are needed.

Many laboratories have implemented the turbidometric method, introducing different experimental flavors in the process. However, these assays are most useful when only estimates of solubility are needed. The analysis of solution turbidity must be done within minutes of preparation, because solutions do not remain turbid for long, and precipitates form. These precipitates can fall out of the path of the light source and go undetected, leading to false negatives.

Additionally, turbidometric work is prone to the mixing effect, in which compounds form gums or resins when introduced into aqueous solution. Such an effect can block the flow cell or contaminate other samples when gums or resins formed in mixing chambers are not adequately removed.

Nephelometry—the measurement of light scattered by a suspension in plate format—was recently introduced as another particle detection technique (*4*). Like turbidometry, nephelometry uses a standard curve to determine the concentration at which a compound comes out of solution. It also requires fast analysis of compounds and can only be used to obtain the kinetic solubility. It too depends on clear-bottom plates that are free of imperfections and is affected by problems such as gumming and resin formation.

Concentration-based assays

In concentration-based assays, a compound is added to buffer or water in a 96- or 384-well plate, in a quantity sufficient to form a precipitate, and the concentration of material that remains in solution is determined by spectroscopic methods (*5, 6*). These assays differ from the particle detection methods in that solutions must be homogeneous for accurate analysis. Typically, samples of stock solution are introduced into a solvent and incubated in a multiwell plate. Samples are then filtered to make them homogeneous and are analyzed. Some methods use calibration curves to determine concentration, but it is possible to accurately determine concentration from only a single reference spectrum. Most experimenters favor UV spectroscopy because most compounds normally have some UV absorbance, but other methods such as chromatographic quantitation are possible.

With these assays, there is no concern about the time interval between sample introduction and measurement. In fact, quite the opposite is true—long incubation times are encouraged, and equilibrium conditions can be established. Because hundreds of samples are processed in parallel, the method produces results at high-throughput speed. Thus, it is possible for concentration-based assays to obtain data that correlate with thermodynamic measurements (Figure 3). Unlike particle detection, concentration-based measurements can foreshadow problems in development and provide key information to chemists down the pipeline (*1*).

The future

Chemists now realize that it is not good enough to make active compounds; rather, they must start thinking about the end product earlier. This has led to an increase in the use of solubility studies to support drug discovery (*3, 6, 7*). Researchers are beginning to favor concentration-based measurements over simple particle detection. This change might be due to the reproducibility of the measurements and the flexibility to examine multiple pH levels. But it has also become important to justify the instrumentation development and validation cost with downstream value. Unlike particle detection methods, concentration-based solubility assays can be used to assess solubility–pH profiles important for flux prediction, to determine intrinsic solubility, and to look at solubility in different media or with alternative excipients. Obtaining this information early may be key to the success of some programs. As Lipinski recently stated at the 2003 North Jersey ACS meeting (*8*), insufficiencies in "aqueous solubility will continue to be a major impediment to achieving oral activity."

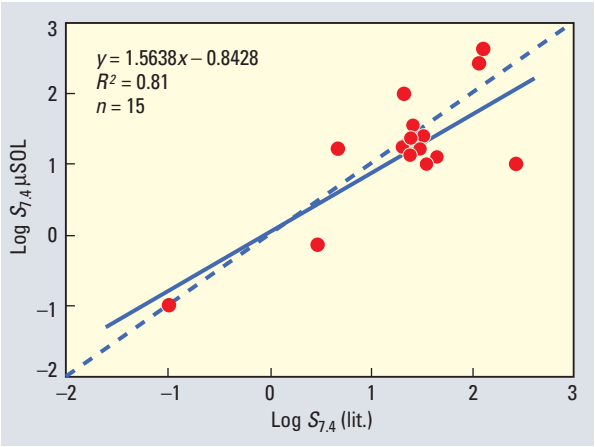

Figure 3. Thermodynamics and solubility. A comparison between published thermodynamic solubility results and µSOL, a concentration-based solubility analysis performed under equilibration conditions containing <1% DMSO. (Dashed line represents complete agreement.) (Figure courtesy of *p*ION Inc.)

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