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Thermodynamic equilibrium solubility measurements in simulated fluids by 96-well plate method in early drug discovery ☆

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ABSTRACT

An early prediction of solubility in physiological media (PBS, SGF and SIF) is useful to predict qualitatively bioavailability and absorption of lead candidates. Despite of the availability of multiple solubility estimation methods, none of the reported method involves simplified fixed protocol for diverse set of compounds. Therefore, a simple and medium-throughput solubility estimation protocol is highly desirable during lead optimization stage. The present work introduces a rapid method for assessment of thermodynamic equilibrium solubility of compounds in aqueous media using 96-well microplate. The developed protocol is straightforward to set up and takes advantage of the sensitivity of UV spectroscopy. The compound, in stock solution in methanol, is introduced in microgram quantities into microplate wells followed by drying at an ambient temperature. Microplates were shaken upon addition of test media and the supernatant was analyzed by UV method. A plot of absorbance versus concentration of a sample provides saturation point, which is thermodynamic equilibrium solubility of a sample. The established protocol was validated using a large panel of commercially available drugs and with conventional miniaturized shake flask method (r^2 >0.84). Additionally, the statistically significant QSPR models were established using experimental solubility values of 52 compounds.

Lipinski et al.[1] caution that solubility is a decisive and key parameter for drug discovery. It is wise to solve solubility insufficiencies at discovery stage via structural modifications of a lead candidate so that chemistry can move from a pool of poorly soluble, orally inactive compounds towards those with some degree of oral activity. The understanding of physicochemical and solid-state properties of drug substance provides the basis to develop suitable dosage form. However, before this, it is prerequisite to know thermodynamic solubility so that the drug substance and its performance can be optimized.[2–7] Drug penetration through lipid membrane depends on lipophilicity of the molecule and thus, may be correlated with partition coefficient value. Absorption of drug depends on the correct balance between these two opposite properties.[8] A lack of solubility affects the ability of drug to achieve efficacious and toxicologically relevant exposures in animals. This parameter also affects future developability and formulation efforts for a lead candidate.[9,10]

The acceptance criteria and solubility classifications for discovery project teams and medicinal chemists propose that <10 μg/mL are considered to be low soluble, 10–100 μg/mL as moderately soluble and compounds with solubility >100 μg/mL are considered to be highly soluble.[11] This classification range is projected to provide general guidelines on potential solubility issues for human oral absorption. However, this criterion usually is too low for animal dosing in solution formulation. Based on these classifications, compounds with aqueous solubility of >100 μg/mL are unlikely to show solubility related issues in further development. However compounds with solubility between 1 and 100 μg/mL may require formulation development in order to overcome poor absorption issues associated with their low solubility. Compounds with even lower solubility typically represent a real formulation challenge.[12,13] In spite of these known facts, solubility problems are often recognized for the first time when an oral solid dosage form or parenteral formulation has to be developed. This late identification of poor solubility is attributable to the extensive use of organic solvents at early stages of drug discovery and even in the first animal experiments. By the time formulation development starts, it is usually no longer practical to chemically change the active compound. Unfortunately, attempts to save drug candidate through suitable formulation are expensive, time-consuming and not always successful. Therefore, solubility of new substances should be determined as early as possible and is a critical step in assessing likely developability of a compound.[1]

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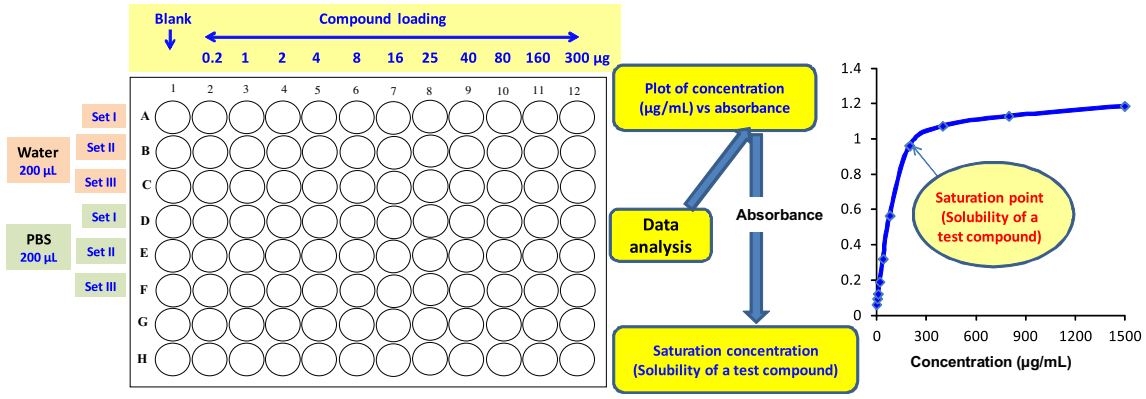

Figure 1. Experimental design for determination of thermodynamic aqueous solubility of compounds.

There exists variety of approaches for solubility determination at different phases of drug discovery and development including in silico approaches and experimental solubility (kinetic or thermodynamic). Measuring thermodynamic equilibrium solubility is considered as the 'gold standard'.[14,15] Kinetic solubility measurement involves predissolution of compound in a co-solvent [generally in DMSO (dimethyl sulfoxide)] followed by the precipitation after dilution in a suitable solution.[16] However, the use of co-solvents leads to overestimation of solubility values.[17–20] Thermodynamic solubility is helpful in diagnosing in vivo results as well as it serves as a guide for formulation development and for regulatory submissions. Thermodynamic equilibrium solubility experiments involve addition of an excess of solid compound to dissolution medium. The equilibrium concentration of a compound in solution is determined at the end of dissolution process. This is often considered as the 'true' solubility of compound and is generally measured using shake-flask method. This method is labour-intensive and when performed in test tubes or vials, requires a large amount of compound. Thus, solubility experiment can be ongoing for several days due to long equilibration times.[21,22] Among all available literature methods for solubility determination of compounds, most of them are for kinetic solubility.[19,23–25]

For measuring thermodynamic aqueous solubility, shake flask method is routinely used approach, which requires large amount of sample, as it uses high volume of dissolution medium. Therefore, the shake-flask approach is not suitable in early drug discovery lead optimization stage. Although miniaturized shake flask methods have also been established,[12,26] still these protocols require significantly large amount of sample (~100 mg), which is practically not amenable to medicinal chemistry lead optimization. Furthermore, during early development stage, large numbers of compounds/NCEs (new chemical entity) are synthesized with very small quantities (<50 mg). To address sample quantity challenge, it is highly desirable to develop an accurate, reliable, fast and miniaturized solubility platform for measuring solubility, which would require little amount of compound with improved throughput. Some efforts have been made to establish such protocols,[27,28] however it is important to mention that these protocols suffer from certain drawbacks, which limit their utility in early drug discovery, where amount of sample synthesized is limited. Heikkila et al.[27] reported protocol in which varying amounts of drug has been loaded in wells. The amount of drug loading in wells was different for different drugs, ranging from 0.19 to 300 mg of drug per test medium (in triplicate) and the concentration of stock solution is inconsistent for each compound. Thus, in this protocol, the sample requirement is highly variable and requires 0.76–1200 mg sample for 4 test media (in triplicate). Furthermore, the use of varied volume of stock solution for each sample reduces throughput of the reported method. Therefore, this protocol does not provide general guidelines for NCEs, and thus it cannot be used routinely in medicinal chemistry lead optimization. Roy et al.[28] reported protocol in which 3 mg of a sample was directly introduced (as a solid) into a well, and the solubility was determined using calibration curve method. Using this protocol, the sample requirement for estimation of solubility in four media (in triplicate) is 176 mg. If these protocols are to be used for a new compound, some idea of its probable solubility is required.

At early drug-discovery stage, there is a necessity to establish unvarying protocol for measuring thermodynamic solubility so that even NCEs can also be straight-a-way analyzed. This uniformity in the protocol is in the terms of amount of sample requirement, strength of stock solution used for the experiment and sample loading into wells. Therefore, the aim of this study was to develop uniform and widely applicable 96-well plate protocol (with UV detection) for determination of aqueous thermodynamic equilibrium solubility [in water, PBS (phosphate buffer saline), SGF (simulated gastric fluid) and SIF (simulated intestinal fluid)] and validate it by conventional shake flask method. The developed protocol overcomes above mentioned limitations for measuring thermodynamic solubility in a medium-throughput manner. The time required for solubility determination in all four test medium is approximately 5 h per sample which is much less as compared to that of conventional shake flask method where time for equilibration is 24 h for each test medium. The added advantage is that total of only 10 mg compound is required to

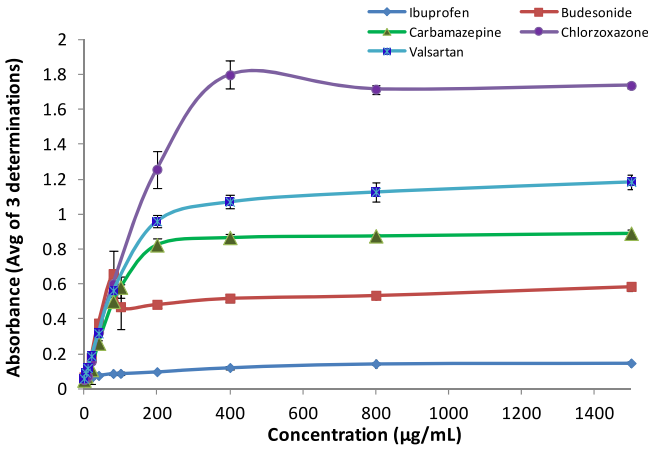

Figure 2. Solubility profile of ibuprofen, budesonide, carbamazepine, chlorzoxazone and valsartan in water at 25 °C.