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Bridging solubility between drug discovery and development

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Solubility has a crucial role in the success of a drug candidate. Compounds with low solubility not only cause problems for *in vitro* and *in vivo* assays, but also add significant burdens to drug development. Drug discovery and drug development often have different solubility screening requirements and methodologies have been developed to meet the needs of these different stages.

Solubility issues in drug discovery and development

Low solubility of new chemical entities (NCE) is emerging as a major issue in drug discovery and development. Poorly soluble compounds not only create problems for *in vitro* and *in vivo* assays in drug discovery, but also place a significant burden on drug development. Compounds with insufficient solubility have a higher risk of attrition and higher costs in drug development. Recent studies showed that 75% of the drug development candidates had low solubility and belonged to Biopharmaceutical Classification System (BCS) classes II and IV [1]. Hence, there is a significant increase in insoluble NCEs when compared with current marketed drugs. The increase of low solubility compounds in discovery portfolios is associated with several factors. Firstly, many hits of drug discovery programs are now routinely obtained through high throughput screening (HTS) of compound collections. These collections are typically made up of legacy compounds from previous projects, file enrichment activities and compound purchases. Typically, the latter two classes have been employed to increase size and diversity of the collection but might suffer from a bias towards lower solubility due to the chemistries and purification methods employed. HTS hits tend to have higher molecular weight (MW) and higher lipophilicity, which contribute to lower solubility [2,3]. A molecule with high MW requires higher energy both to create a bigger cavity and to insert into the solvent phase, resulting in lower solubility. During lead optimization, more lipophilic substituents are added to the hits to improve potency and selectivity, which often further decreases solubility. Secondly, there is a shift of therapeutic targets from aminergic G-protein-coupled receptors (GPCRs) and enzymes to more challenging ones, such as kinases, ion channels, nuclear receptors and protein–protein interactions [4,5]. These types of target classes often require more lipophilic compounds for affinity [6] or compounds with strong intermolecular interactions, such as wide aromatic regions and/or intermolecular hydrogen bonds, leading to high crystal-lattice energies, which often lowers solubility. Hence, these changes in the drug discovery process and therapeutic targets have resulted in a trend towards increasing low solubility NCEs. The higher number of insoluble NCEs in the discovery phase might be, at least in part, attributed to the misconception that solubility can always be fixed later using pharmaceutics approaches (e.g. formulation), unlike other properties that highly rely on chemistry optimization (e.g. cytochrome P450 inhibition, hERG).

The challenges associated with decreased solubility vary with the disease area. For example, solubility is often less of an issue for neuroscience projects, but frequently a major issue for oncology programs. Compounds intended for neuroscience diseases typically require high potency (low nanomolar activity) to minimize potential side-effects, and many neuroscience compounds contain basic amines and can be ionized to increase solubility. Also, for central nervous system (CNS) drugs, blood–brain barrier penetration is needed, which makes high MW less favorable. By contrast, for oncology NCEs, achieving sufficient solubility is often a major hurdle. Compounds for treating oncology tend to have high doses and often require intravenous (IV) formulations (i.e. the full dose is preferably dissolved in injection volume). Many current oncology projects target protein kinases, and their inhibitors tend to

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have planar templates with high lipophilicity, multiple aromatic rings and a network of hydrogen bond donors/acceptors, leading to low solubility. This is because multiple conjugated aromatic rings with planar templates tend to have strong π–π interactions between molecules and create tight crystal packing. Strong networks of intermolecular hydrogen bonding require high energy to break them and bring the molecules into solution. Solubility issues are often project-specific and driven in part by therapeutic targets.

During the past 15 years, the pharmaceutical industry has recognized the importance of solubility and developed several strategies to either improve candidate druggability or overcome poor solubility using solubilization techniques [1,2,7]. The approaches include applying *in silico* methods during structural design to predict solubility risk, screening solubility to identify potential issues early, modifying structure to improve solubility, and developing formulations to increase solubility and dissolution rate. Drug discovery and development have similar yet distinct solubility applications and objectives. It is important to develop methodologies and strategies that meet the solubility needs and bridge the differences between drug discovery and development.

Solubility in drug discovery

Solubility information is widely used in drug discovery to enhance the quality of drug candidates, help project teams make informed decisions and increase the success rate in the clinic [2,8].

Solubility impact on preclinical in vivo *studies*

Solubility is an important parameter for oral absorption. Insoluble compounds contribute to poor oral absorption and poor oral bioavailability. They are more susceptible to food effects and often exhibit large individual variability for *in vivo* exposure. Besides solubility-limited absorption, oral absorption can also be dissolution-rate-limited. If compounds have a slow dissolution rate, they might miss the window (1.5–4 hours depending on the species [9]) of absorption in the small intestine and have reduced fraction absorbed (F_a). Therefore, improving the dissolution rate can help enhance oral absorption of dissolution-rate-limited compounds. Supersaturation (drug concentration in solution is above solubility) can affect *in vivo* oral absorption as well, because *in vivo* intraluminal concentration is much higher than equilibrium solubility. For example, the hydrochloric acid salt of a farnesyltransferase inhibitor, FTI-2600, was found to have 400% greater plasma concentration in dogs compared with the free base after oral dosing, owing to a fivefold increase in dissolved concentration compared with free base [10]. This suggests that technologies that induce supersaturation are beneficial for successful development of insoluble compounds. Equilibrium solubility is an important parameter; however, it is not the only parameter affects *in vivo* oral absorption. A holistic physiologically based pharmacokinetics (PBPK) model that incorporates the complex solubilization processes and parameters in the gastrointestinal (GI) compartments (e.g. equilibrium solubility, dissolution, precipitation and supersaturation) is more effective in predicting oral absorption accurately [11]. Early formulation development of insoluble compounds is essential to enable *in vivo* pharmacokinetics (PK), efficacy and toxicity studies. Frequently, generic formulations are employed for a fast turnaround time. For example, 0.5% methylcellulose in water and 0.1–2% Tween 80 with 0.5% methylcellulose in water are some of the most commonly used formulations for oral administration in drug discovery. Generic formulations are not optimal, because they are not designed or optimized based on the specific physiochemical properties and stability of each compounds. Several effective approaches have been developed to formulate and deliver insoluble compounds in drug discovery with limited material and short timelines for *in vivo* PK, efficacy and toxicity studies [12–16]. In some cases, solubilizing insoluble compounds can require special vehicles [17]. Some excipients can interfere with *in vivo* studies and lead to confounded data and might not be tolerated upon multiple dosing [18,19]. For example, some surfactants used in drug formulations are P-glycoprotein (P-gp) inhibitors [20] and they might enhance oral absorption and brain penetration of P-gp substrates and alter their *in vivo* PK profiles, depending on the administration route and the relative doses of the drug and the excipients [21]. Hence, precautions should be made when selecting excipients, their quantities and regimens for formulation to minimize *in vivo* impact from dosing vehicles. When adequate aqueous solubility or formulation preparation do not translate to adequate systemic exposure, solubility data are crucial to diagnose the potential causes. Solubility data can be used to simulate *in vivo* PK, which might aid prediction for efficacious doses, especially solubility in intestine fluids (e.g. FaSSIF and FeSSIF). Solubility in physiological fluids can be used to anticipate potential precipitation issues after intravenous (blood or plasma) or oral dosing (GI fluids) [16].

Importance of solubility in bioassays in vitro

Solubility has a crucial role for the success of *in vitro* absorption, distribution, metabolism, excretion and toxicity (ADMET) and bioassays. Insoluble compounds tend to give erratic and erroneous assay results, and cause confusion for project teams [8,22]. Low solubility in DMSO due to freeze–thaw processes can also cause a large percentage of compounds having lower concentration than targeted [23]. Compounds with poor aqueous and dimethyl sulfoxide (DMSO) solubility usually lead to artificially weak potency and low HTS hit rates [24]. Sometimes, a clean profile in a counter screen for off-target activities can be due to low solubility of the compound in the assays [22]. The data can be misinterpreted as having good selectivity, and an inferior compound could be identified for further advancement for flawed reasoning. Therefore, early solubility information is beneficial to guide compound selection, help decision-making and provide an early alert for potential issues in ADMET and bioassays. Optimization of bioassay protocols compatible with the accurate evaluation of low solubility compounds is an important achievement towards successful assay development. Several approaches have been developed to overcome solubility challenges for *in vitro* bioassays including [8,22]: avoiding intermediate aqueous dilution, maximizing DMSO content, using cosolvents, screening at lower concentrations, performing serial dilution in organic solvents and using real-world drug discovery compounds in assay development. In one reported case, addition of 2-hydroxypropyl-β-cyclodextrin (HBC) and surfactants (e.g. Pluronic F-127) to HTS assay plates was found to be beneficial for increasing recovery and the robustness of the assays [25]. Properly using additives in bioassays can enhance assay quality. Some of the commonly used pharmaceutical excipients for *in vivo* studies are now being used for *in vitro* bioassays to overcome solubility issues. Solubility information is also useful to

help identify compounds for more resource consuming studies, such as in structural biology using X-ray or nuclear magnetic resonance (NMR) for ligand–protein interaction studies to increase the chance of success in these investigations.

Solubility applications in medicinal chemistry

The design of small molecule NCEs suitable for clinical development is challenging because medicinal chemists need to balance multiple crucial parameters (e.g. target potency, minimal off-target activity, ADMET, safety properties, efficacy in preclinical models, patentability and biopharmaceutical properties) into a single molecule. Ideally NCEs should have sufficient solubility to ensure that adequate drug exposures can be confidently predicted and then achieved in preclinical pharmacokinetic, efficacy and toxicology studies to ultimately underwrite clinical candidate selection [26]. Several approaches have been developed to improve solubility of initial screening hits during the lead optimization phase of drug discovery projects, and some successful strategies are discussed below.

In silico predictions

Developing predictive *in silico* solubility models for real NCEs are useful for medicinal chemistry optimization, structure–property relationships, in projecting the solubility impact on preclinical/clinical PK, and in foreseeing the risk of development candidates. Many computational models have been developed to predict solubility [27–29] and several commercial software products are available [30]. The major challenge in accurately predicting solubility is that it is difficult to predict crystal packing [27]. If the solubility of a compound is mostly governed by lipophilicity, it is relatively easier to predict solubility with good accuracy. However, if the solubility of a compound is mostly controlled by crystal packing, it is much more difficult to predict accurately. The quality of experimental data is also crucial for model development, because it is commonly referred to as 'garbage in, garbage out' (bad data in, bad data out). Various experimental limitations (e.g. nonspecific binding to filter and apparatus, incomplete phase separation and slow equilibrium) can lead to significant errors in prediction. Models developed using non-drug compounds are less useful to predict solubility of real NCEs, because they have different chemical space. Global models (based on wide chemical space) are usually less predictive than local models (based on a small chemical space such as a compound series); however, they are useful for general compound ranking. Local models require more resources to develop for specific projects, but they can predict solubility with greater accuracy [31]. Typically, both types of models are applied in parallel depending on project needs and resources available. Validated predictive models of solubility built from quality data sets (ideally solubility data aligned with an assessment of solid form) can be applied to ensure that proposed NCEs have a good probability of improved solubility. Furthermore, a pairwise analysis of the impact of single point changes in molecular structure on solubility has been determined with a large collection of diverse molecules such that beneficial (and detrimental) modifications can be reasonably predicted [32].

Structural modifications

Improvements in solubility of small molecule NCEs can often be achieved through structural modification by disruption of molecular planarity and symmetry to reduce crystal packing [33] and/or by introducing solubilizing groups at positions on the core structure of a lead series without being detrimental to other properties [30]. For example, the use of alkoxy amines tethered to the core pharmacophore has been successfully applied in the discovery of calcium channel blockers (amlodipine) [34], estrogen receptor modulators (tamoxifen) [35] and farnesoid X receptor (FXR) agonists with a significant increase in solubility over the original lead [36]. In the FXR example, the lead compound FXR-450 had low solubility (0.014 mg/ml) in the standard suspension formulation with 2% Tween 80 with 0.5% methylcellulose (dosing vehicle) and led to low oral bioavailability in mouse (21%, Fig. 1). Through structural modification, by introducing a basic amine as an ionizable center and a floppy tail to disrupt the crystal packing, a new candidate was identified with much improved solubility (0.25 mg/ml neutral form, 5.92 mg/ml HCl salt form in dosing vehicle) and

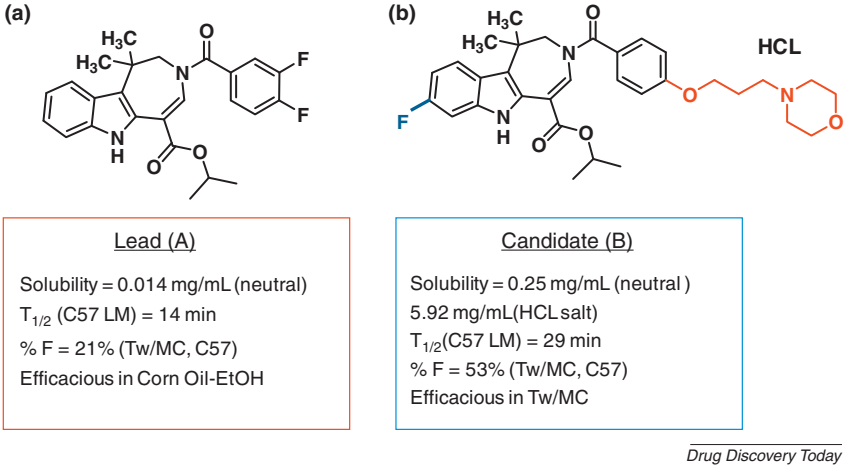

FIGURE 1

Structural modification to enhance solubility [36]. The lead compound (A) has poor solubility leading to low oral bioavailability and it only demonstrated efficacy in oil based formulation. Structural modification improved solubility and oral bioavailability. Candidate (B) is efficacious in the standard aqueous suspension formulation with Tw/MC. *Abbreviations*: C57: C57BL6 mouse; LM: liver microsomes; Tw/MC: Tween 80/Methylcellulose formulation; %F: percent bioavailability.

led to high oral bioavailability (53% in mouse). Improved metabolic stability of the candidate also contributed to the increased in oral bioavailability. Structure–solubility relationships are frequently developed to guide solubility improvement [30].

Salt form approaches

When an NCE contains an ionizable group (cationic or anionic) there will be an opportunity to identify a preferred salt form with improved biopharmaceutical properties over the parent molecule. Appropriate salt forms can increase dissolution rate and lead to faster and more complete oral absorption [shorter T_{max}, higher C_{max} and AUC (area under the curve)]. An example using salt form to increase oral bioavailability has been demonstrated by piroxicam [37]. Piroxicam salts showed significant increase in dissolution rate and oral bioavailability compared with piroxicam [37]. In selecting counter-ions for salt screening, it would be prudent to prioritize counter-ions with some degree of clinical precedence for the desired route of administration [38]. Pharmaceutically acceptable counter-ions are listed in several references [30,39].

Prodrug strategies

Prodrug approaches are frequently applied to improve solubility of insoluble compounds, enhance oral absorption and enable safe and effective IV formulations [40,41]. Phosphate ester prodrugs have clinical precedence (e.g. fosfluconazole, fosphenytoin and fosamprenavir) where solubility has been increased by orders of magnitude over the parent drugs. Phosphate ester prodrugs rely on alkaline phosphatase enzymatic cleavage in the gut lumen or blood to release the parent drug. When considering a prodrug strategy, it is important to consider the potential toxicity liability caused by the release of the prodrug-solubilizing auxiliary from the prodrug. Other types of water-soluble prodrugs include using amino esters, polyethylene glycol (PEG) esters and others [40–42].

Solubility in drug development

In drug development, the solubility of the active pharmaceutical ingredient (API) is a crucial attribute with respect to: (i) selecting the dosage forms for clinical trials; (ii) designing experiments to identify potential salt forms, cocrystal forms, polymorphic forms, solvates and hydrates; (iii) developing analytical procedures and (iv) aiding drug manufacturing strategies. The philosophy to assess solubility applications at the preformulation stage has been included in the concept of 'General Pharmaceutics' [43].

Solubility in predicting human PK and dose

Solubility information for an API is useful as input in predicting human oral PK and in designing dose regimens in clinical trials. Solubility data in simulated intestinal fluids are important for predicting *in vivo* oral PK using PBPK models [44], such as GastroPlus™ and PK-Sim®. It was found that using solubility data in aqueous buffer tends to underestimate absorption (providing lower predicted C_{max} and AUC) of low soluble compounds compared with using solubility data in simulated human intestinal fluids [44]. Bile salt and lecithin in simulated intestinal fluids can enhance solubility and dissolution rates of lipophilic compounds and improve drug absorption [45]. Simulated gastric fluids are acidic and can dissolve basic compounds leading to higher drug concentration in the intestine than equilibrium solubility due to supersaturation [26,46]. Simulated GI fluids are more physiologically relevant than solubility in pure buffers and therefore give better *in silico* prediction. To successfully simulate *in vivo* PK, *in silico* models need accurate solubility data to predict the dynamic processes of a compound in the GI tract (dissolution, precipitation, salt form, among others, throughout the GI pH gradient) [47,48]. For early drug discovery and development projects intended for oral delivery, maximum absorbable dose (MAD) might be used to estimate the amount of compound absorbed from the intestine [49,50]. If the MAD is lower than the projected efficacious dose, formulations can be applied to improve solubility.

Solubility in designing clinical dosage forms

To develop a clinical dosage form for development, it is preferable that any formulation used during preclinical PK studies be transferable to clinical formulations by the same administration route. For clinical dosage form development, solubility in various potential formulation vehicles is determined to identify the best possible approaches, including aqueous solubility in multiple pH buffers (solubility-pH profile), and solubility in various excipients, such as cosolvents, cyclodextrins, surfactants and oils. The excipient quantity needs to have acceptable tolerability based on the intended route of administration. For some projects, special techniques are used to formulate insoluble compounds depending on the physicochemical properties, such as: amorphous solid forms [51], sprayed dried dispersions (SDDs) [52], self-emulsifying drug delivery systems (SEDDS) [53], micronization and nanoparticle approaches [54]. Cocrystals are emerging as a solid form option for non-ionizable pharmaceuticals that have deficiencies such as oily or amorphous nature, stability issues or low solubility/poor dissolution rate [55,56]. Cocrystals are formed through specific non-covalent interactions between the NCE and one or more ligands (coformers) all of which are neutral and solid at room temperature. Cocrystals can significantly enhance the biopharmaceutical properties of certain NCEs as illustrated by the use of a glutaric acid cocrystal to improve oral bioavailability and low solubility of a $NaV_{1.2}$ blocker [57]. The success stories of using cocrystals to increase solubility are still rare and time will tell the impact of this technology on improving solubility and oral exposure.

Solubility impact on clinical trial design

Compounds with high permeability/lipophilicity and poor solubility tend to have positive food effects [58–60], whereas compounds with low permeability and high solubility often show negative food effects [61]. Solubility in fasted-state simulated intestinal fluid (FaSSIF) and fed-state simulated intestinal fluid (FeSSIF) solutions can help predict potential food effects *in vivo* and formulations might be used to minimize food effects [62]. Food effects can be due to several reasons, such as delayed gastric emptying, increased gastric pH, slower input into the intestine, stimulation of bile salt secretions, increased blood flow, and competition for metabolizing enzymes [63,64]. Formulations can be used to minimize food effect and create more consistent

exposure across individuals. Clinical trials can be designed to evaluate the effect of food on drug absorption/metabolism and the results can impact product labels (http://www.cerep.fr/Cerep/Users/pages/ProductsServices/GPCRPlatform.asp).

Solubility in BCS and biowaivers

Solubility and permeability are the two parameters for establishing the appropriate BCS class (U.S. Food and Drug Administration, The Biopharmaceutics Classification System (BCS) Guidance, http://www.fda.gov/AboutFDA/CentersOffices/CDER/ucm128219.htm). Compounds are classified into four classes based on solubility and permeability [65]. BCS is described in the 'Guideline for Industry' by the US Food and Drug Administration (US FDA) for biowaivers of immediate release (IR) oral products [66]. Biowaivers can be requested for BCS class I compounds in IR products. If a product meets the criteria for solubility, *in vitro* dissolution profile, stability in the GI tract, quality of excipients, therapeutic range, among others, the product is considered bioequivalent to the reference product without the need for human pharmacokinetic studies.

Solubility in organic solvents

The solubilities of an API in organic solvents are useful information for solid form screening (i.e. crystallization, salt forms, cocrystals, polymorphism, hydrate and solvates), purification, formulation development, process development, analytical chemistry, manufacture and cleaning validation [67]. Software, such as COSMOTherm® (http://www.cosmologic.de/index.php?cosId=4203&crId=4), can be used to rank-order organic solvents and pharmaceutical excipients based on solubility to narrow the design space and enhance the chance of success.

Measuring solubility

Many solubility methodologies have been developed over the years to measure solubility, including kinetic, semi-equilibrium (close to equilibrium) and equilibrium methods [30,68]. Typically more than one solubility method is applied to support the different stages of drug discovery and development within an organization. Selected solubility methods used in various pharmaceutical companies and legacy Pfizer (http://www.pfizer.com/home/) companies are summarized in Tables 1 and 2. Typically a kinetic or semi-equilibrium method is employed in early drug discovery as a high throughput (HT) assay to profile a large number of compounds, and medium throughput (MT) and/or low throughput (LT) equilibrium methods are employed for later stages of drug discovery and development. Samples for high throughput methods often start as a concentrated DMSO stock solution. The DMSO stock solution is either added directly into solubility buffer, or dried by evaporation to leave the compound and then buffers are added to the remaining material to minimize the effect of DMSO on solubility measurement. The solution is either measured right away based on light scattering of the precipitate (kinetic method) or incubated for about one day before filtration and measurement of the concentration (semi-equilibrium method). The amount of DMSO in the solubility assays varies from 0 to 5% and the pHs of the buffers are typically pH 6.5 (intestine) or pH 7.4 (bioassay and blood). The different analytical tools used for detection (e.g. light scattering/turbidity, UV plate reader, LC-UV and

TABLE 1

Selected high throughput published solubility methods in the pharmaceutical industry

Companies	Method type[a]	Methods	Buffer	DMSO stock (mM)	Target concentration (μM)	DMSO (%)	Ref
AstraZeneca	Semi-equilibrium	UV plate reader	pH 7.4	10	100	1	[74]
Boehringer Ingelheim	Kinetic	Nephelometric	pH 7.4	10	250	2.5	[75]
GSK	Kinetic	Nephelometric	pH 7.4	10	500	5	[76]
Novartis	Semi-equilibrium	LC-UV	pH 1.0 pH 6.8	10 evaporated	1000	0	[77]
Roche	Semi-equilibrium	UV plate reader	pH 6.5	10 evaporated	1000	0	[68]

[a] Semi-equilibrium solubility is measured after long incubation to enable time for equilibrium to be established between solid and solution. Solubility values tend to approaching equilibrium solubility.Kinetic solubility is measured after adding DMSO stock solution into a buffer and no incubation is involved. Supersaturation is common in kinetic solubility measurement and it is typically higher than equilibrium solubility.

TABLE 2

High throughput solubility methods used in legacy Pfizer companies

Legacy Pfizer companies (location)	Method type[a]	Buffer	Target concentration	DMSO (%)	Ref
Pfizer (Groton, CT, USA)	Kinetic	pH 7.0	65 μg/ml	0.05–0.65	[2]
Warner–Lambert	Kinetic	pH 6.5	200 μM	2	[78]
Pharmacia	Semi-equilibrium	pH 3, 7, 10	250 μM	2.5	–
Pfizer (Sandwich, Kent, UK)	Kinetic	pH 7.4	200 μM	5	–
Wyeth	Semi-equilibrium	pH 7.4	100 μg/ml	1	[79]
Pfizer (Nagoya, Japan)	Semi-equilibrium	pH 6.5	300 μM	1	[70]
Pfizer (Groton, CT, USA)	Semi-equilibrium	pH 6.5	600 μM	2	–

[a] Semi-equilibrium solubility is measured after long incubation to enable time for equilibrium to be established between solid and solution. Solubility values tend to approaching equilibrium solubility.Kinetic solubility is measured after adding DMSO stock solution into a buffer and no incubation is involved. Supersaturation is common in kinetic solubility measurement and it is typically higher than equilibrium solubility.

TABLE 3

Advantages and limitations of common detection methods for solubility measurement

Detection methods	Advantages	Limitations
Light scattering/turbidity	Universal, fast, economical	Interference from certain colored compounds and impurities, sensitive to sedimentation and particle size, low sensitivity, measures precipitates rather than solution concentration
UV plate reader	High sample coverage, fast, economical, sufficient sensitivity for solubility measurement, good linearity over wide dynamic range	Requires UV chromophore, interference from impurities and matrix material
LC-UV	High sample coverage, less interference from impurities and matrix material, sufficient sensitivity for solubility measurement, good linearity over wide dynamic range	Requires UV chromophore, might need different HPLC methods for special compounds, not as fast and economical as UV plate readers
LC–MS	High sensitivity, high selectivity, low interference	Less universal, moderate sample coverage, low dynamic range for linearity, too sensitive for solubility measurement (need large dilution), high maintenance, costly
LC-CLND	No standard curve needed	Only for nitrogen containing compounds, interference from nitrogen containing solvents, significant signal loss for adjacent nitrogen

Abbreviation: CLND: chemiluminescent *nitrogen detection*.

LC–MS/LC-CLND) can sometimes impact assay results. Table 3 highlights the advantages and limitations of the common detection methods for solubility measurement. Understanding of the limitations of each detection technique is crucial for proper data interpretation and for selecting alternate analytical tools as appropriate. The target solubility (Tables 1 and 2) varied over a wide range from 100 μM to 1000 μM, reflecting the different focus of various organizations. Many project teams concentrate on improving solubility of insoluble compound series (10 μg/ml), because this will have a great impact on absorption. Covering the low solubility ranges in a high throughput assay is crucial to guide medicinal chemistry design to enhance solubility. However, it is also important to set the target solubility high enough to cover high dose compounds. For compounds with average potency and permeability, a solubility of 50–60 μg/ml will give good oral absorption in human [3] and, therefore, a target solubility of 100 μM is sufficient for projecting oral absorption. By contrast, for compounds that are not very potent (e.g. 10 mg/Kg dose) and not very permeable, the solubility requirement for good oral absorption is much higher (500–2000 μg/ml) [3]. For this reason, the maximum solubility of the assay is typically set higher to anticipate potential absorption issues for high dose compounds with low permeability. Because DMSO increases solubility, assays that use stock compounds in DMSO have a limited maximum solubility, owing to the specified % DMSO in the assay and the DMSO stock concentration. The solubility targets for discovery compounds that will translate into sufficient solubility for development candidates have been discussed in detail [3,49], and they are dependent on potency and permeability of the compounds. The high throughput solubility methods are intended to measure a first estimation of solubility, and so to help define the ADMET space of the series and anticipate potential issues. These results are by no means the definitive solubility values of a compound used in support of final candidate registration.

It is difficult to propose one high throughput solubility method suitable for all applications because each method has its relative merits and flaws. Beyond the technical aspects of the HT screening method, other important factors will be the willingness of an organization to invest in creating (or sourcing) the screening platform and the skill of the project team in using this data

TABLE 4

An example of tier approach for solubility screening

Methods	HT solubility	MT solubility	LT solubility
Sample source	30 mM DMSO stock	Powder	Powder
Buffers	pH 1.2, 6.5, 7.4	SGF, pH 6.5, FaSSIF	SGF, pH 6.5, FaSSIF, FeSSIF
Replicates	$N = 1$	$N = 1$	$N = 1$–3
Target solubility	600 μM	300 μg/ml	3 mg/ml for SGF 300 μg/ml for others
Incubation time (hour)	18	24	24 and 48
Incubation temperature	RT	RT	37°C
Detection	UV plate reader	LC/MS, PLM	LC/MS, PLM/PXRD
Throughput	High	Medium	Low
Timing	Early drug discovery	*In vivo* oral PK	Candidate selection

Abbreviation: RT: room temperature; PLM: polarized light microscopy; PXRD: powder X-ray diffraction; SGF: simulated gastric fluid; *N*: number of replicates.

TABLE 5

Comparison of solubility between non-crystalline and crystalline material [16,69–71]

Compounds	Solubility ratio of non-crystalline/crystalline
Ivermectin	1
Lansoprazole	1
Simvastatin	1
Trichlomethiazine	1
Hydrochlorthiazide	1.1
Griseofulvin	1.4
Efavirenz	1.4
Sulfamethoxydiazine	1.5
Diclofenac	2.6
Indomethacin	4.5
Caffeine	5.0
Polythiazide	9.8
Tamoxifen	11
Tolnate	18
Theophylline	50
Morphine	270
Benzimidazole derivatives	500–1000

appropriately. Our recommendation is to use a low cost, high throughput solubility assay early in the drug discovery process and bridge with more detailed studies as compounds advance through the pipeline. For mid to late stage drug discovery compounds, more detailed MT/LT solubility assays are then applied. Solubility of development candidates in physiological fluids (e.g. FaSSIF, FeSSIF) are measured to more accurately predict absorption potential in the clinic.

One major distinction between high throughput kinetic/semi-equilibrium and low throughput equilibrium methods is the crystal form determination. Crystal form affects solubility of a compound and amorphous material is usually more soluble than crystalline material. Table 5 shows the solubility comparison of amorphous material versus crystalline material of a selected set of compounds. The solubility of amorphous material can be 1–1000-fold higher than crystalline material and it is compound dependent [16,69–71]. Solubility of different crystalline polymorphic forms or hydrates can differ by two- to tenfold [13,72]. Although crystal form has a crucial role in solubility, it is often not determined in early drug discovery solubility assays due to several reasons. Most drug discovery compounds are obtained from evaporation of solvents quickly using rotary-vacuum, which often generates amorphous material. Even if the material is crystalline, the crystal form is not characterized at this stage. Furthermore, most of the compounds are dissolved in DMSO and stored in compound banks for dispensation for various uses. This process erases any memory of crystal form information. Most solubility methods for early drug discovery screening use DMSO stock solution. Even for methods which dry down DMSO, the solid materials are often amorphous or have unknown crystal forms. The drying process eliminates the DMSO solubility-enhancing effect, but not crystal packing. A majority of compounds are likely to present in amorphous form under the common high throughput solubility conditions. Crystal, salt form and purity profile are likely to change throughout the drug discovery processes. Crystal form determination has limited value at this early point in the screening funnel when sourced from DMSO stock solutions. It is better to align crystal form information with solubility when the first dry solid sample is prepared. High throughput solubility presents the 'best case scenario' (i.e. highest solubility), because of the solubility enhancement effects of DMSO, potential non-crystalline material and impurities. If compounds have low solubility in the amorphous state, it can only get worse as crystallinity and purity increase. Therefore, if a compound has low solubility in a high throughput solubility screen (the 'best case scenario'), it is probable that solubility might be an issue for the compound. Strategies should be developed to address solubility issues early. When NCEs are dosed as a dissolved solution, polymorphic crystalline forms have minimal impact on oral PK. By contrast, if undissolved suspensions are used for oral study, different polymorphic forms, which have different solubilities, will have different oral absorptions and, therefore, different oral PK characteristics. Project teams should measure equilibrium solubility as crystalline materials become available to gauge the differences compared with kinetic solubility. At candidate selection stage, in-depth solid form characterization is recommended to assess risks due to potential solid form changes during formulation development, manufacturing and clinical trials. An example of a commonly used solubility screening paradigm employed at Pfizer is shown in Table 4.

Bridge solubility needs between drug discovery and development

Integrated solubility approaches have been developed to meet the needs of drug discovery and development [68,73]. A schematic diagram of the alignment of different solubility screening methods with the discovery and development stages is shown in Fig. 2. *In silico* and high throughput solubility screenings have been used to support early drug discovery programs from hit identification, hit-to-lead to lead optimization processes. The early solubility information helps to diagnose bioassay problems, provides early alerts on potential solubility issues, and guides structural modification to improve solubility. Medium throughput equilibrium solubility assays are applied during lead optimization and candidate selection when solid materials are available to assess the risks associated with oral absorption and to diagnose *in vivo* pharmacology and PK issues. The more accurate equilibrium solubility data generated at this stage is used to verify the solubility data obtained from high throughput methods to gauge the differences between the methods and develop a path forward on whether to continue to screen with HT methods, or to change to MT equilibrium methods for a project. Low throughput equilibrium solubility measurements are performed for late stage development candidates to provide detailed information on the formulation strategies and overall profile of the candidate developability. Polymorphic form of the residues from solubility measurement is fully characterized at this stage with X-ray powder diffraction, DSC, TGA, polarized light microscopy, Raman spectroscopy and other techniques. Experimental solubility data generated using various HT, MT and LT methods are useful to develop and refine *in silico* models to predict solubility under the assay conditions. As

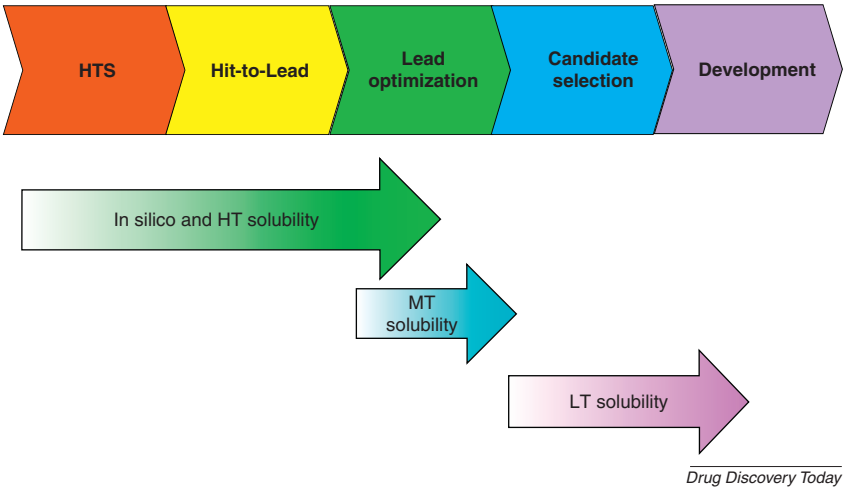

FIGURE 2

Solubility screening strategies. In silico and HT solubility methods can be used to screen compounds at early stages of drug discovery from HTS, hit-to-lead to lead optimization. MT solubility assays are applied during lead optimization and LT solubility measurements are used to support candidate selection and drug development. *Abbreviations*: HT: high throughput; HTS: high throughput screening; LT: low throughput; MT: medium throughput.

the experimental data are being generated, they can be updated in the models on a regular basis to continuously improve the quality of prediction.

Traditionally, low solubility was considered a drug development issue and it was addressed late in the drug discovery process by pharmaceutical scientists through formulation approaches. By then, it was often too late to modify the molecule to improve solubility or select a different candidate with better solubility. As a consequence, projects failed due to issues caused by low solubility, such as: (i) poor *in vivo* exposure leading to marginal efficacy, (ii) narrow therapeutic index (TI) caused by limited exposure in dose escalated toxicity studies, (iii) expensive or unstable formulation, or (iv) severe food effect. Based on the learning in the 1980s and early 1990s, the pharmaceutical industry started to address solubility issues early in the drug discovery processes and strategies have been implemented to improve solubility and bridge the solubility gaps between drug discovery and development [2]. High throughput solubility methods are widely applied to provide an early alert to potential solubility issues. Structure–solubility relationships are developed to guide medicinal chemistry to improve solubility through structural modification. *In silico* models are built to predict solubility and oral absorption. The early solubility approaches in drug discovery revolutionized how the pharmaceutical industry addressed ADME issues and contributed to the reduction of attrition. However, addressing solubility issues in early drug discovery also creates an interesting dynamic. Because HT solubility values are usually different (typically higher) than LT equilibrium solubility, for the reasons discussed above, it can create controversy among project teams on what numbers to believe and how to use them. For example, compounds might have good solubility in the HT assay or *in silico* prediction, but might be confirmed later to be insoluble in the LT equilibrium solubility assay with crystalline material. This can lead project teams to become more skeptical about the ability of HT and *in silico* methods to estimate solubility accurately. Similarly, compounds might be shown to have low equilibrium solubility, but have high oral absorption *in vivo* when salt forms or formulations are used. These controversies can cause confusions and frustration among project teams. In the solubility world, there are no 'one size fits all' approaches. Bridging studies are often needed, based on project needs, to close the solubility gaps between drug discovery and development. Selected compounds can be additionally assayed using a LT equilibrium solubility method to check the offset between the kinetic or semi-equilibrium solubility and equilibrium solubility values. As needed, the LT method can be used earlier for specific drug discovery projects with particular needs. By the same token, dissolution rate and supersaturation might be determined for development candidates and the information might be incorporated into PBPK models to accurately predict oral absorption and PK. High quality solubility data measured during drug development are continuously fed back to drug discovery to enhance the *in silico* models and improve predictability. Education of end users is crucial in transitioning solubility information from drug discovery to development and vice versa, so that a unified approach is applied throughout the entire process.

Concluding remarks

Solubility affects many aspects of drug discovery and development, including: *in vitro*/*in vivo* assay quality, formulation, human PK, clinical trial design and biowaivers. Insoluble NCEs continue to increase in incidence owing to the use of HTS and the nature of modern drug targets. Strategies have been developed to overcome solubility issues using medicinal chemistry approaches and formulation techniques. Solubility assays are not 'one size fits all' and different methods with varying throughput are applied at different stages of drug discovery and development. Tier solubility approaches are being adapted throughout the pharmaceutical industry to de-risk the solubility impact on the drug candidate success.

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