Themed Issue: Enabling Drug Developability - Challenges in Optimizing Biopharmaceutical Properties of New Drug Candidates
Guest Editor - Sae-ho Chong

Discovery Pharmaceuticals—Challenges and Opportunities

Submitted: March 17, 2006; Accepted: March 29, 2006; Published: June 2, 2006

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ABSTRACT

Most pharmaceutical companies are now evaluating compounds for druglike properties early in the discovery process. The data generated at these early stages allow upfront identification of potential development challenges and thus selection of the best candidates for lead nomination. Most often, lead nomination candidates are selected based on pharmacological and toxicological data. However, many drugs in development suffer from poor biopharmaceutical properties due to suboptimal physicochemical parameters. The poor biopharmaceutical properties often lead to extended timelines and a higher cost of developing the compounds. To avoid these problems and choose the best compounds from a biopharmaceutical perspective, physicochemical parameters such as solubility, lipophilicity, and stability need to be evaluated as early as possible. Furthermore, the preformulation approaches used to evaluate the compounds for their pharmacokinetic and toxicological properties need to be optimized. This minireview summarizes some of the parameters and approaches that can be used to evaluate compounds in the early stages of drug discovery.

KEYWORDS: Physicochemical properties, stability, solubility, preformulation

INTRODUCTION

The main reasons for attrition in drug discovery and development are safety or tolerability concerns, poor absorption, disposition, metabolism, and excretion (ADME) properties, and lack of efficacy. In an effort to reduce these attrition rates, most pharmaceutical companies are now evaluating their lead molecules for druglike properties much earlier in the discovery process. The data generated at this early stage allow upfront assessment and identification of development challenges and provide the possibility of optimizing druglike properties at the lead optimization stage, thus enabling the selection of the best candidate for lead nomination.

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Even though pharmaceuticals alone is not a major attrition factor, many drugs in development are affected by their poor biopharmaceutical properties. When a compound with suboptimal biopharmaceutical properties is selected for development, considerably more time needs to be spent on formulation and process development, ultimately leading to higher costs and delays. Selection of lead compounds that have the appropriate physicochemical properties and adequate chemical and physical stability, and that can readily be formulated to give appropriate bioavailability from a relevant dosage form, can ultimately lead to faster development timelines, reduced cost, and diminished complexity of the development process.

The objective of this article is to review the pharmaceutical properties of drugs that can be evaluated in drug discovery and how their evaluation can enable the selection of compounds with appropriate properties and fewer development challenges. Furthermore, the rational selection of formulations that can be used in preclinical efficacy, pharmacokinetic, and toxicology studies will be discussed.

PHYSICOCHEMICAL PROPERTIES

Solubility

The aqueous solubility of a drug substance is a fundamental property that should be evaluated early in discovery. Lack of solubility can affect the results of early high-throughput screening assays and the ability to achieve efficacious and toxicologically relevant exposures in animals. This characteristic will also affect the future developability of and formulation efforts for the compound.

Solubility depends on the solvation energy of the solute in the solvent overcoming both the crystal lattice energy of the solid and the energy to create space in the solvent for the solute. Thus, the solubility of a compound depends not only on properties of the drug molecule itself, such as polarity, lipophilicity, ionization potential, and size, but also on properties of the solvent and the solid, such as the crystal packing and presence of solvates.¹

Solubility measurements throughout discovery range from methods that dilute dimethylsulfoxide (DMSO) stock solutions in aqueous buffers and mimic the methods in which high-throughput assays are run, to those measuring pseudo-equilibrium solubility using crystalline solids and aqueous

buffers. Detection methods include turbidimetric methods, UV plate readers, liquid chromatography (LC)/UV, and LC/mass spectrometry (MS). The solubility method chosen depends on the desired turnaround time, the quantity of compound, and the quality of the results required. For more details on these methods, the reader is referred to several general references²⁻⁵ and additional specifics that can be found in other recent reviews.⁶⁻⁸

In the early stages of discovery, when many compounds are synthesized in small quantities for high-throughput screening, high-throughput solubility methods can provide an early rank-ordering that can help weed out the lowest-solubility compounds, which would be expected to give rise to the highest development hurdles. These methods typically are those that involve dilutions starting with the compounds dissolved in DMSO.^{3,5-7}

As compounds move to later stages of discovery, a more reliable assessment of solubility is required. These solubility measurements should ideally start with the solid compound, preferably crystalline, and the experiments should run for a long enough time that they approach equilibrium.

A crystalline form is desired as compounds get closer to lead selection, because although the crystalline form available in discovery may not be the final form chosen for development, the likelihood that the solubility of 2 crystalline forms differs by more than ~2 fold is slight, whereas the difference in apparent solubility between an amorphous and a crystalline form of a compound can be many orders of magnitude.^{9,10} The crystallinity of a sample can be assessed by polarized light microscopy, or, if enough compound is available, a more detailed characterization can be obtained by powder x-ray diffraction and thermal analysis.¹¹

Ionization Constant (pK_a)

For a compound containing basic or acidic functional groups, solubility at a given pH is influenced by the compound's ionization characteristics. The solubility of a compound in aqueous media is greater in the ionized state than in the neutral state. Thus, solubility of ionizable compounds is dependent on the pH of the solution. Many drugs are weak acids or bases and thus are ionizable within the pH range of the gut. Solubility and dissolution, and therefore absorption, of a weak base can be altered by changes to gastric pH (eg, when coadministered with antacids). While a weakly basic compound might fully dissolve in the acidic environment of the stomach and result in high exposure levels under such conditions, coadministration of drugs that raise the stomach pH can lead to greatly decreased solubility, leading to significantly lower exposure. Other considerations for an ionizable compound include the impact of ionization on stability and permeability and for

compounds containing both acidic and basic functional groups the formation of zwitterions, for which the solubility at the isoelectric point is typically the lowest over the entire pH range.

If a compound is ionizable, a salt can be formed, which may have better dissolution or an improvement in other physical properties such as hygroscopicity, solid-state stability, or the potential for polymorphism. The solubility product, K_{sp} , needs to be taken into consideration in predicting the solubility of a salt in a particular environment that contains other salts with a common counterion.

There are many experimental methods used in a discovery setting for determining the pK_a . Simple fitting of the pH-solubility profile can be used if solubility measurements have already been made at multiple pH values. Other typical methods include potentiometric titration, spectrophotometric titration, and capillary electrophoresis.^{12,13}

Lipophilicity

Lipophilicity affects solubility and permeability as well as other ADME properties such as protein binding and tissue distribution. The partitioning of a compound is dependent on its ionization state and thus is dependent on pH. The reader is referred to Comer and Tam for more details about lipophilicity.¹⁴

The most commonly used method to measure lipophilicity is the shake-flask method to determine the octanol/water partition coefficient. After an adequate equilibration time, the concentration of compound in an octanol layer (or another lipophilic phase) and the concentration in an aqueous layer are measured. More recently, this method was adapted to a 96-well plate format by Analiza (Cleveland, OH). Additionally, high-performance liquid chromatography (HPLC) methods have been developed.¹⁵

Surface Activity

Many drugs are surface active, that is, they have both a hydrophilic part and a hydrophobic part and are therefore capable of micelle formation. Surface activity can increase solubility in aqueous media because of micelle formation or other aggregation, where the hydrophobic groups of the molecule are not exposed to the aqueous environment. Surface-active compounds can also disrupt membranes and can thus lead to toxicity.¹⁶

If the solute is a hydrophobic drug molecule, addition of surfactants to aqueous solvent can increase solubilization by incorporating the solute into micelles. This solubilization effect can be seen with simulated gastrointestinal fluids, which typically contain bile salts or other surfactants.

The surface tension of solutions of surface-active compounds, as well as their critical micelle concentration, can

be measured using several different techniques. These methods include detachment methods such as DuNuoy ring and Wilhelmy plate, maximum bubble pressure, and pendant drop. More details on these methods can be found in physical chemistry textbooks such as Adamson and Gast.¹⁷

STABILITY

The stability screening of drug candidates plays an important role in drug discovery. The goal of a stability study at this stage is to obtain an overview of the stability of the compound in a variety of pharmaceutical situations and therefore to identify potential liabilities that may affect drug development. The information obtained from these studies can be used to (1) provide feedback to the research team for modification of the labile groups to improve stability; (2) help the development scientists to determine the developability of the compound; (3) provide guidelines on compound handling and storage; and (4) provide information to guide stabilization strategies.^{2,3,8}

The revised parent drug stability test guideline Q1A (R2) issued by the International Conference on Harmonization (ICH) requires that the drug substance be tested under different stress conditions that are indicative of environmental challenges to which the drug will be exposed. It is suggested that stress testing include the effect of pH, temperature, humidity, light, and oxidizing agents.¹⁸

To generate high-quality data, a robust, stability-indicating assay needs to be developed. Frequently, a reverse-phase HPLC assay that allows direct injection of stability samples suffices. While not always practical at the discovery stage, an ideal assay should allow detection of degradation peaks equivalent to 0.1% of the parent peak, which is consistent with the ICH impurity guidelines. This level of detection allows the quantitation of the appearance of degradation products instead of the disappearance of the parent compound, which provides a more precise measurement of degradation.^{19,20}

In recent years, automated systems capable of performing and analyzing multiple degradation experiments on drug substances under various stress conditions have been reported.^{21,22} These automated workstations can provide structural and kinetic information on the degradants during the experiment. They also are much faster than manual approaches and are amenable to high-throughput measurements in a 96-well format.

pH-Dependent Stability in Solution

Information on the stability of a compound in solution is needed to understand its characteristics under physiological conditions and to develop solution dosage forms. The pH-dependent stability of a drug substance can be tested at 37°C

at pH 1, pH 4, pH 7, and pH 9 for 1 day up to 1 month by HPLC analysis. The studies should be initiated at a reasonable concentration to detect even minor decomposition products in the range of detection. For compounds with low solubility, organic solvents such as acetonitrile and methanol can be added.

The hydrolytic degradation of a compound in acidic and alkaline conditions can be studied by refluxing the drug in 0.1N HCl and 0.1N NaOH for 8 to 12 hours. If the drug is found to degrade completely, both the time and the temperature of the study can be decreased.²³

Solid-State Stability

The solid-state stability of drug substances as a function of temperature and humidity should be studied at the candidate selection stage. Solid-state reactions that occur in drug substances include solid-state phase transformation, dehydration/desolvation, and chemical reactions.²⁴ Solid-state degradation of pharmaceuticals is often related to molecular mobility, and common degradation pathways include oxidation, cyclization, and hydrolysis.

The chemical stability of a drug substance in the solid state can be evaluated under various temperature and humidity conditions. Preweighed samples are stored in stability cabinets at 40°C, 60°C, 25°C/85% relative humidity (RH, open vials), and 40°C/75% RH (open vials) for 2, 4, and 8 weeks. At predetermined time intervals, samples are removed, dissolved in appropriate solvent, and analyzed by HPLC.

It is usually difficult to assess the solid-state phase transformation during discovery because of the limited amount of drug material. Solids from the above-mentioned studies should be evaluated at the end of the experiment by differential scanning calorimetry (DSC), thermogravimetric analysis, and powder x-ray diffraction to determine whether there are any changes in polymorphic forms across the conditions tested. Preliminary results obtained from this study can help the development scientists to design full-scale solid-state stability tests.

Oxidative Stability

Oxidation is one of the most common degradation pathways for organic compounds. Oxidation can occur through chain processes that involve initiation, propagation, and termination steps and are catalyzed by heat, light, metals, or free radicals. It can also occur via electron-transfer reactions to form reactive radicals of anions or cations. In the solid state, oxidation occurs where molecular oxygen diffuses through the crystal lattice to the labile sites.^{25,26}

Certain functional groups show particular sensitivity toward oxidation. For chain processes, oxidative degradation is

linked to the lability of hydrogen atoms within the molecular framework. For example, substituted aromatics such as toluenes, phenols, and anisoles are susceptible to hydrogen abstraction because the aromatic group can stabilize the resulting radical through resonance. For electron transfer reactions, nitrogen (amines), sulfur heteroatoms (sulfides, disulfides, and sulfoxides), and oxygen-based anions (phenol anions) are common sites for electron-transfer-induced oxidation, producing final products such as N-oxides, sulf-oxides, sulfones, and ketones.²⁶

At the drug candidate selection stage, oxidative stability can be tested in solution in the presence of 100-200 ppm hydrogen peroxide or other free radical initiators such as 2,2'-azobis(2-amidinopropane) dihydrochloride. While it may not be necessary and is rather difficult to test solid-state oxidative stability in discovery, a recent report by Simon et al demonstrated a rapid method that may be useful for rank-ordering the oxidative stability of solids. In this method, parameters describing the lengths of oxidation induction periods were obtained from nonisothermal DSC measurements based on the dependence of onset temperature of the oxidation peak on heating rate.²⁷

Photostability

Exposure of a drug to irradiation can influence its stability, and a test of a compound's photostability should be performed at the discovery stage. The information about the compound's photoreactivity is needed to provide information for handling, packaging, labeling, and use of the drug substance or product.²⁸

At the candidate selection stage, photostability should be evaluated both in the solid state and in solution. For solid-state photostability, the preweighed drug substance is stored at high-intensity light (HIL)/UV conditions at 25°C in a photostability chamber according to the ICH guidelines (1.2 million lux hour exposure to visible light and 200 W hour/m² to UV). At the end of the experiment, the solid is dissolved in appropriate solvent and analyzed by HPLC. For solution photostability, solutions at appropriate concentration are stored at ICH HIL/UV conditions and analyzed by LC. For both solid and solution photostability, samples protected from light are stored under the same condition and used as controls.

Mechanisms of Degradation

While it is not always feasible in drug discovery to identify all degradation products and elucidate degradation mechanisms, preliminary studies on these aspects will guide development scientists in solving the stability problems and designing a strategy for stabilization. Traditional methodologies, which use spectral and elemental analysis, are often

slow and resource-consuming. The modern approach is to integrate systems in which LC/MS or LC/MS/MS is employed to obtain molecular weight and fragmentation information or to use LC/nuclear magnetic resonance to obtain further detailed structural information.²⁹ The integrated approach provides rapid and unambiguous identification of several degradation products at one time.

PREFORMULATION

Formulation Challenges in Discovery

Vehicle selection for preclinical in vivo studies can be a major challenge for discovery scientists. In preparing, scientists must determine the objective of the in vivo studies since the type of constraints placed on the vehicle can be very different for different objectives. For example, if the goal of the study is to get an initial idea of the oral efficacy of the compound, it is essential that the excipients selected for the vehicle not interfere with the measured end points. The easiest way to get this initial evaluation of efficacy may be to use solution formulations rather than suspensions and in that way reduce the complexity of the studies. If, on the other hand, the main goal of the in vivo study is to get an idea of whether the compound is developable, a more complex crossover design using both solution and suspension dosing may be needed. This section will further discuss the use of solution and suspension vehicles.

Solution Vehicles

In selecting a solution vehicle the solubility of the compound in aqueous and nonaqueous systems is determined first to identify the various vehicle possibilities. At the same time, the chemical stability in these solvents should be evaluated. Several strategies can be used to increase the solubility of compounds with low aqueous solubility. If the compound is ionizable, it may be possible to achieve the target concentration through pH adjustment. If pH adjustment does not give the desired results, a cosolvent can be used. Polyethylene glycol 400 (PEG 400) is a good example of a commonly used inert cosolvent that has wide application across several therapeutic areas for both oral and parenteral administration.³⁰ If the in vivo model for preclinical pharmacology testing precludes the use of vehicles such as PEG 400 and propylene glycol, then other, more exotic vehicles such as N-methyl-2-pyrrolidone, Labrafil, and vitamin E TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate) may be considered. In other instances a complexing agent such as cyclodextrin can be used to increase the compound's solubility. Care should be taken to ensure that compounds are soluble in cyclodextrin solutions at the target concentration, especially when the discovery program has vast diversity in their chemical structures. Otherwise,

instead of screening compounds for biological efficacy it is possible that the efficacy study is inadvertently screening for the ability of the compound to form cyclodextrin complexes.

It is often desirable to give a drug parenterally in solutions at a concentration that exceeds its aqueous solubility. For intravenous administration, there are numerous approaches that may be adopted for solubilizing the compound.³¹ Use of cosolvent is most often the preferred route to increase the solubility of these compounds. When this approach is used, care has to be taken not to exceed toxicity levels for the cosolvent. Furthermore, the formulation may cause hemolysis or the drug may precipitate out of the formulation immediately after injection. Yalkowsky and his group have developed an in vitro precipitation model to predict the potential of compounds to precipitate upon intravenous administration.^{32,33} This simple model can be used to predict whether precipitation of a compound might occur on dilution or injection.

Suspension Dosing

An important part of candidate selection is the design of pharmacokinetic (PK) studies that explore potential problems and define the probability of success of formulation approaches to enable future clinical studies. An early evaluation of potential formulation options aids in the assessment of factors that could lead to increased development time and costs and can be critical for decision making and prioritization of development resources.

Most drugs are developed for oral administration of a solid dosage form. The impact of solubility and dissolution rate of the solid drug substance on the rate and extent of oral drug absorption should, therefore, be evaluated and compared with the absorption achieved after solution administration. Because of limited compound availability and time constraints that are usually experienced in discovery settings, a simple suspension formulation is most often chosen for this purpose. Crystalline material should be used for this suspension dosing, since amorphous drug is likely to have significantly higher solubility and a significantly higher dissolution rate in the gastrointestinal (GI) tract, potentially leading to unrealistic absorption levels that cannot be reproduced once a crystalline form of the drug has been identified at a later stage of the development process.

In a case where dissolution of the solid drug is much faster than absorption of the dissolved drug into the body, permeability, not dissolution, is the rate-limiting step for absorption. Suspension and solution dosing should result in the same systemic exposure levels in such a case. However, more commonly the dissolution of the solid drug proceeds slowly, and once the drug is dissolved it readily permeates through

the gut wall. Here, drug absorption is therefore dissolution-rate-limited, and dosing a suspension will result in significantly reduced exposure compared with solution dosing. Any changes in dissolution rate that can be achieved through formulation approaches, such as reduction of particle size of the solid drug or administration of a salt in the case of an ionizable compound, can lead to a profound effect on the rate and degree of drug absorption. If, however, the solubility of the drug in the GI fluid is extremely low and saturation of the intestinal fluid with the drug is achieved at low concentrations, reduction of particle size will not have the desired effect of increased exposure. Absorption in this case is not dissolution-rate-limited but solubility-limited. Which of these 2 phenomena is responsible for low drug absorption after administration of a suspension often depends on the dose administered. At low doses, saturation of the GI fluid with drug might not be reached, and a decrease in particle size might result in increased systemic exposure, indicating dissolution-rate-limited absorption. An increase in dose at a given particle size will result in dose-related increases of systemic exposure up to a certain level. Above this level, drug concentrations in the GI fluid reach saturation, and a further increase in dose or reduction of particle size will not lead to increased exposure. In this scenario, drug absorption changes from being dissolution-rate-limited to solubility-limited and exposure levels reach a plateau, indicating the need for more complex formulation technologies such as the generation of high-energy, amorphous drug substance or the design of a solubilized, precipitation-resistant formulation to overcome solubility-limited absorption.

In vivo crossover studies during drug discovery for comparison of the performance of suspension and solution formulations are especially important during the evaluation of potential candidates with low aqueous solubility. An early idea of the highest exposure that can be reached from a simple suspension dosing of a given drug candidate and how this exposure compares to projected efficacious levels in humans is essential for the early identification of potential development hurdles. Once these data have been obtained, suitable approaches and adequate resources can be planned when the compound is moved into development to minimize surprises during clinical development.

Formulation for Toxicology Studies

Development of vehicles for preclinical toxicology testing for discovery compounds is often very challenging. Toxicology studies, in general, cover a wide range of doses (3- to 100-fold or greater over the target dose), and this range makes it extremely challenging to develop formulations with inert vehicles that can also provide high exposures as the dose is increased. Achieving dose-proportional increases in exposure is almost impossible in cases where

the target therapeutic dose is high (>1 mg per kg of body weight). If, on the other hand, the dose is low, then there are multiple options for toxicology formulations and the likelihood of achieving high exposures is increased.

Low aqueous solubility of the compounds under physiologically relevant pH conditions often precludes the development of solution formulations in purely aqueous vehicles. Available options are suspension formulations, which might lead to reduced exposure, especially at higher doses, or non-aqueous vehicles. The challenge with nonaqueous vehicles is the potential that high levels of a given vehicle might not be tolerated by the animals over a prolonged period of time. Another consideration during the development of a vehicle for toxicology studies is the chemical stability of the test compound in the vehicle over the use time.

CONCLUSIONS

At the discovery stage the process of simultaneously optimizing lead molecules for their pharmacokinetic properties, physicochemical properties, toxicology, selectivity, and potency can lead to compromises in one or more of the properties an ideal drug candidate should have. Often the compromise is made in the area of achieving optimal physicochemical properties (eg, solubility, lipophilicity), resulting in the lead candidate having suboptimal biopharmaceutical properties. It is essential to get an initial idea of the physicochemical and biopharmaceutical properties of the compounds and to evaluate what type of formulation effort would be needed to rectify any problems. Based on the results from these studies, a decision will then have to be made about whether to move the compound forward or to try to find a better candidate.

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