Methods in Pharmacology and Toxicology

Springer Protocols

Gary W. Caldwell
Zhengyin Yan *Editors*

Optimization in Drug Discovery

In Vitro Methods

Second Edition

Humana Press

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ISSN 1557-2153 ISSN 1940-6053 (electronic)
ISBN 978-1-62703-741-9 ISBN 978-1-62703-742-6 (eBook)
DOI 10.1007/978-1-62703-742-6
Springer New York Heidelberg Dordrecht London

Library of Congress Control Number: 2013953234

Printed on acid-free paper

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Springer is part of Springer Science+Business Media (www.springer.com)

Preface

The discovery and commercialization of new drugs for humans is extremely complex. Typically, pharmaceutical companies have approached this pharmacology challenge by dividing the problem into three stages. These stages in a pharmaceutical drug development process are the discovery of drug candidates interacting at a particular therapeutic target using whole-animal models, the development of these drug candidates into new chemical entities (NCEs) using human subjects, and the commercialization of NCEs into medicines. The process requires an enormous financial investment since a decade or longer is typically required to transform a drug candidate into a medicine. In addition and probably most important is that the process requires a large interdisciplinary team of scientists and support staff working closely together with a focused management team to be successful.

Whole-animal *in vivo* pharmacology models, which are required by drug regulatory agencies, are the gold standard for biopharmaceutic, pharmacokinetic, toxicokinetic, and pharmacodynamic late stage drug candidate predictions; however, for many biological pathways and mechanisms, they do not provide a good extrapolation to humans. To address this issue, pharmaceutical scientists have used an *in vitro* and *in situ* surrogate assay reductionisms approach to understand biopharmaceutic, pharmacokinetic, toxicokinetic, and pharmacodynamic properties and thus to select drug candidates that have a high probability of becoming an NCE and eventually a medicine (Fig. 1). These surrogate assays provide more representative methods to rule out adverse effects early in the screening process for new drug candidates and to provide a knowledge platform for the correlation of whole-animal *in vivo* pharmacology results to humans. In this strategy, surrogate assays have been developed to understand the biopharmaceutics of drug candidates including the solid-state characteristics of the drug in physiological fluids; that is, dissolution rates, dissociation constants, ionization potential, lipophilic partition coefficients, hydrophobicity, stability, solubility, formulation, and permeability. The pharmacokinetics and toxicokinetics of drug candidates have been addressed by examining individual physiological processes such as absorption (i.e., passive, active, efflux transport of drugs), distribution (i.e., tissue, protein, and cell drug binding), metabolism (i.e., cytochrome P450 (CYP) and UDP-glucuronosyltransferases (UGT)), and drug excretion mechanisms (i.e., metabolism, renal and bile). The overall pharmacodynamic predictions of the drug candidates have been rationalized by receptor binding and functional assays and safety assessment assays including CYP inhibition and induction, drug–drug interactions via assessment of reactive metabolites, hERG (the human *Ether-à-go-go*-Related Gene), DNA damage, genotoxicity, and mutagenicity assays.

Thus, based on this reductionism approach, the pharmacology of a drug can be understood and examined by studying its parts, such as biopharmaceutics, pharmacokinetics, toxicokinetics, and pharmacodynamics. As previously mentioned, each sub-part contained in Fig. 1 can be further subdivided into *in vitro* and *in situ* surrogate assays. Due to the

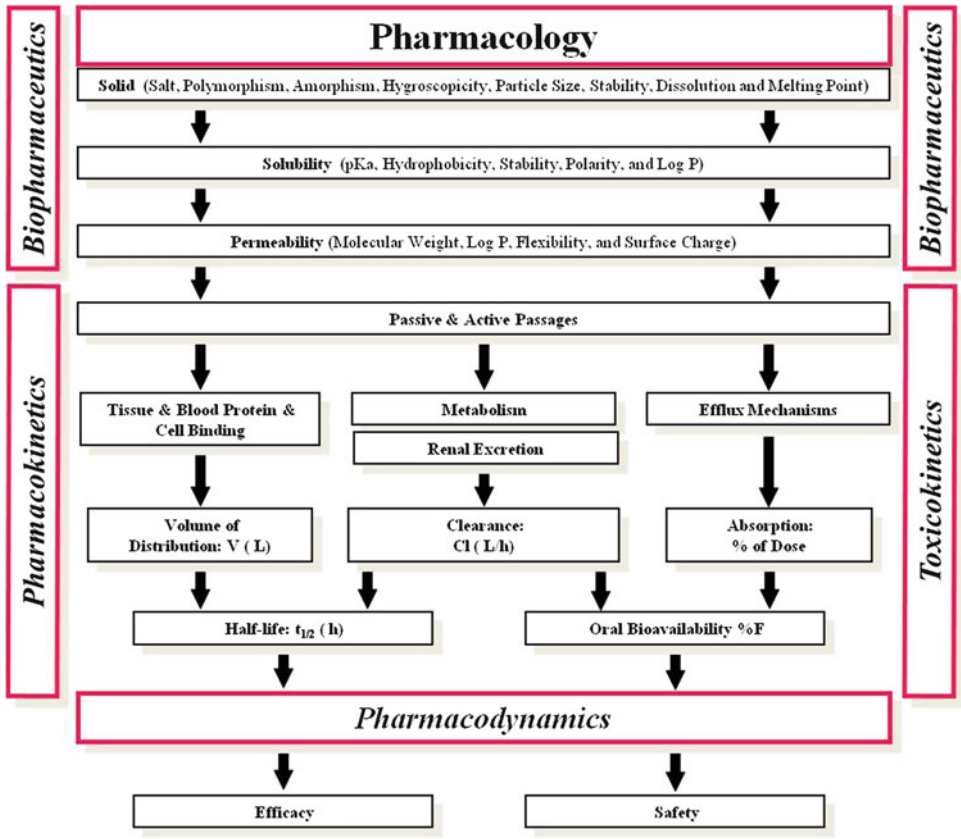

Fig. 1 Pharmacology of a drug

large number of drug candidates that need to be tested, drug discovery and development groups in the pharmaceutical industry have adopted an assay tiered approach toward selecting potential new drug candidates with superior drug properties from large compound collections; that is, funneling thousands of compounds through a series of high-throughput capacity assays to lower capacity assays, which reveal more and more detailed information on a particular sub-part of the reductionism scheme. Using this approach, "drug-like" characteristics in addition to efficacy properties and good safety profiles are achieved. With this process in mind, the book, ***Optimization in Drug Discovery: In Vitro Methods***, first published in 2004, presented a compilation of detailed experimental protocols necessary for setting up a variety of *in vitro* and *in situ* assays important in the selection of drug candidates based on the reductionism scheme outlined in Fig. 1. Each chapter contained Introductions, Materials, Methods, Notes, and References sections providing scientists with important background information on the assay, a list of all the equipment and reagents necessary to carry out the assay, a step-by-step protocol, information on dealing with common and unexpected experimental problems in the assay and finally, a listing of important supplementary readings.

We now have compiled a second edition following the same format as the first edition, which contains updated variations on previously reported assays and many new protocols. A total of 34 chapters have been contributed by experts covering a wide spectrum of subjects

including formulation, plasma binding, absorption and permeability, cytochrome P450 (CYP) and UDP-glucuronosyltransferases (UGT) metabolism, CYP inhibition and induction, drug transporters, drug–drug interactions via assessment of reactive metabolites, genotoxicity, and chemical and photo-mutagenicity assays.

Since biopharmaceutic, pharmacokinetic, toxicokinetic, and pharmacodynamic are all interrelated, it has been long recognized that a series of *in vitro* and *in situ* assays are required to understand how to develop "drug-like" characteristics in new drug candidates. For example, biopharmaceutic parameters influence the transfer of a drug across cell membranes, and thus affect absorption and distribution of the drug, which in turn affects pharmacokinetic properties, which in turn affects pharmacodynamic properties and so on. *Chapters 1–3* of the new second edition provide experimental methods for preparing an optimal drug formulation, measuring protein binding and red blood cell binding. When combined with measuring pK_a, solubility, lipophilicity, and plasma protein binding techniques from Chaps. 1, 8, and 9 in the first edition, the most fundamental physicochemical properties of a drug candidate can be determined.

Having a good absorption profile for new drug candidates is another important requirement for a drug to be effective. Drug absorption is primarily governed by solubility properties of the solid neat drug, permeability, and influx and efflux transport mechanisms. *Chapters 4* and *5* are included in the second edition to address different issues on this aspect using a 5-day cultured Caco-2 cell model and an *in situ* single pass perfused rat intestinal model. Combining these assays with absorption models described in Chaps. 2–5 in the first edition, the most commonly used assays to investigate drug absorption mechanisms are available to research scientists.

Optimal metabolic stability of new drug candidates is one property that is necessary for a drug to have a long systemic half-life in the body and thus, lasting pharmacological effects on the action site. There are many different *in vitro* metabolic stability assays that can be used to understand the metabolism fate of new drug candidates, identify potent metabolites with better "drug-like" properties, and for using metabolic stability information to guide new synthesis and generate more stable drug candidates. In *Chaps. 6* and *7* in the second edition, the assessment of CYPs and UGTs metabolism is determined from incubations with either hepatocytes or microsomes. *Chapters 8* and *9* in the second edition outline assays to determine the CYP and UGT phenotyping. When these assays are combined with metabolic stability assays from Chaps. 10–12 in the first edition, an arsenal of assays are available with clear advantages and objectives to address most metabolic stability questions.

Drug–drug interactions (DDIs) are defined when one drug alters the pharmacokinetics or pharmacodynamics of another drug. Since biopharmaceutic, pharmacokinetic, toxicokinetic, and pharmacodynamic are all interrelated, in many cases, one drug generally alters the metabolism or transport of a second drug. Most DDIs involve alternations in the metabolic pathways within the CYP system. There are two mechanisms involve for the CYPs; that is, through the process of CYP induction which increases drug clearance that causes a decline or loss of therapeutic efficacy or when one drug inhibits metabolism of another drug. Drug-CYP induction is typically caused by activation of gene transcription via ligand-activated specific receptor which eventually leads to an increase in CYP enzyme expression. The three most commonly involved nuclear hormone receptors are (1) PXR, which up-regulates expression of the CYP3A, CYP2B, and CYP2C, (2) CAR, which also results in enhanced expression of the CYP3A, CYP2B, and CYP2C, and (3) AhR, which results in enhanced expression of the CYP1A

and 1B enzymes. Nuclear hormone receptor activation assays using stable cell lines are described in *Chaps. 10–13* in the second edition. CYP induction can be evaluated using human hepatocytes as described in *Chap. 14* in the second edition or Chap. 13 in the first edition. Each system has its own advantages and limitations, and the decision to use a particular approach depends upon the goal of the drug evaluation. Drug-inhibition of CYPs is typically caused by reversible and irreversible inhibition mechanisms. In Chaps. 14 and 15 in the first edition, high-throughput screening assays for 13 individual CYPs by using fluorescent substrates and cDNA-expressed enzymes, and 6 individual CYPs using specific substrate probes and human liver microsomes were described to measure reversible inhibition mechanisms, respectively. In the second edition, several assays are described to measure irreversible inhibition mechanisms. *Chapters 15* and *16* in the second edition outline assays to measure irreversible inhibition (i.e., time-dependent inhibition) using plated and suspendered human hepatocytes while *Chaps. 17–19* use human liver microsomes combined with novel detection methods. A systemic approach is given in Chap. 16 in the first edition to identify mechanism-based CYP inhibitors. Thus, a complete set of assays are available to address many DDI questions that occur due to alterations in the metabolic CYP system pathways.

The ATP binding cassette (ABC) superfamily and the solute carrier (SLC) family of transporters play a major role in influencing the pharmacokinetics and toxicokinetics of drugs since they are responsible for the efflux of a plethora of therapeutic drugs, peptides, and endogenous compounds across biological membranes. The ABC subfamily contains nine transporters which have different intracellular localizations, substrate specificities, and structures. In *Chaps. 20–22* in the second edition, *in vitro* methods for discovering substrates and inhibitors for the P-glycoprotein (P-gp/ABCB1), the breast cancer resistance protein (BCRP), and the multidrug resistance-associated protein 2 (MRP2; ABCC2) are discussed in detail, respectively. The organic anion transporting polypeptides (OATPs) are members of the SLC family of transporters. In *Chaps. 23* and *24* in the second edition, *in vitro* assays for discovering substrates and inhibitors of OATP1B1 and OATP1B3, which are predominantly expressed at the sinusoidal membrane of hepatocytes, and OAT1 (SLC22A6), OAT3 (SLC22A8), and OCT2 (SLC22A2), which are primarily expressed in the proximal tubule epithelial cells of the kidney, are discussed in detail, respectively. When these assays are combined with transporter assays from Chaps. 6 and 7 in the first edition, an arsenal of assays are available to understand the major role that transporters play in influencing the drug's pharmacodynamics.

In *Chaps. 25–27* in the second edition, a variety of assays are presented dealing with establishing good *in vitro* LC/MS/MS assays, LC/MS/MS methods for the identification of metabolites in biological fluids, and the detection of endogenous and xenobiotic compounds from biological fluids using LC/MS/MS and dried blood spot techniques, respectively.

The most important clearance pathways for most drugs in humans involve drugs being metabolized by CYP enzymes to more polar compounds that are eventually eliminated in urine. However, CYP enzyme-mediated metabolism can also lead to drug bioactivation resulting in the formation of reactive metabolites that can potentially induce idiosyncratic toxicity by covalently binding to endogenous proteins and nucleic acids before being eliminated from the body. Because reactive metabolites are not stable, direct detection and characterization of them is not technically feasible. Therefore, many assay strategies have been developed to study the bioactivation liability of drug candidates by using trapping reagents that result in the formation of stable adducts which are subsequently characterized

by tandem mass spectrometry. In *Chaps. 28–30* in the second edition, a variety of assays are presented dealing with drug bioactivation including the utilization of trapping reagents that results in the formation of stable adducts, quantitative methods for detecting reactive metabolites using radioactive and non-radioactive reagents, and screening assays for determining the reactivity of acyl glucuronides. When these assays are combined with reactive metabolites assays from Chaps. 24 and 25 in the first edition, a set of assays are available with clear advantages and objectives to help medicinal chemists to optimize lead compounds at an early stage of drug discovery.

The failure of NCEs in both clinical development and aftermarket launch for toxicity reasons is still a major concern for pharmaceutical companies. Therefore, toxicity assays that can provide information at an early stage of drug discovery are of major concerns for medicinal chemists to optimize lead compounds. Interaction of drugs with DNAs potentially results in DNA damage or covalent modifications which may lead to genotoxicity. In *Chaps. 31* and *32* in the second edition, a method for detecting DNA damage at the level of individual eukaryotes induced by drugs using the traditional *in vitro* Comet assay (neutral and alkaline) is presented and a system based in eukaryotic yeast cells that utilize an endogenous DNA damage-responsive gene promoter and a reporter gene fusion to assess the ability of the drugs to damage DNA is presented, respectively. Here the authors provide examples of these assays with detailed procedures used in their laboratory for the analysis and interpretation of assay data. Combining these new versions with DNA damage assays from Chaps. 17–20 in the first edition, a set of assays are available to medicinal chemists to provide a path forward in early stage drug discovery lead optimization programs. Although the Ames test has long been used to detect mutagens and possible carcinogens, an improved version of the assay is given in *Chap. 33* in the second edition as compared to the version in Chap. 21 in the first edition. Also, a new version of the mouse lymphoma assay (MLA) is outlined in *Chap. 34* in the second edition as compared to the version in Chap. 22 in the first edition. In Chap. 23 in the first edition, a high through *in vitro* hERG channel assay was presented.

Finally, we want to express our tremendous gratitude to all the authors that contributed chapters to this book. Without their time and energy, the second edition of Optimization in Drug Discovery: In Vitro Methods would not have been possible.

Spring House, PA, USA — ***Gary W. Caldwell***
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Chapter 1

Small Molecule Formulation Screening Strategies in Drug Discovery

Gary W. Caldwell, Becki Hasting, John A. Masucci, and Zhengyin Yan

Abstract

The correct formulation of new drug candidate compounds in drug discovery is mandatory since the majority of go/no-go decisions to advance candidates are based on *in vitro* ADME, receptor binding, *in vivo* pharmacokinetic and efficacy screens. For this reason, having a rapid formulation screen would be a valuable tool for chemists and biologists working in drug discovery. This chapter will describe a rapid solubilization screen that consumes minimal amounts of compound using an HPLC detection method to measure the solubility of drug candidates in various formulations. Using the pKa and Log P of the compound, formulation selection for drug candidates are based on a decision tree approach to guide the user in the selection of appropriate formulations.

Key words Drug discovery formulation, Solubilization techniques, Buffers, Cosolvents, Surfactants Complexants, Lipids

1 Introduction

The main goal of drug discovery research is to select drug candidates that are worthy of becoming preclinical candidates [1]. These preclinical candidates receive more extensive and time-consuming development in the hope of entering clinical testing. From clinical testing, medicines emerge which are commercialized. This pharmaceutical drug discovery/development process requires an enormous financial investment since a decade or longer is required to take a drug candidate to commercialization [2]. In addition, it requires a large interdisciplinary team of scientists and support staff working seamlessly together with a focused management team.

There is a high attrition rate of drug candidates in preclinical and clinical development due primarily to insufficient efficacy, safety issues, and/or economic reasons. Efficacy and safety deficiencies can be related in part to poor oral absorption, distribution, metabolism and excretion (ADME) properties, pharmacokinetics (PK), toxicokinetics (TK), and formulation issues [3].

Gary W. Caldwell and Zhengyin Yan (eds.), *Optimization in Drug Discovery: In Vitro Methods*, Methods in Pharmacology and Toxicology, DOI 10.1007/978-1-62703-742-6_1,

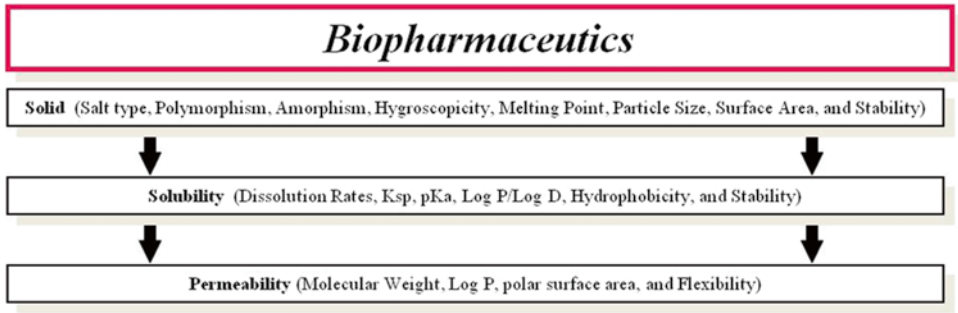

Fig. 1 Factors involved in biopharmaceutics

Therefore, most pharmaceutical companies today use panels of well-characterized ADME/PK, toxicity and formulation screens in parallel with *in vivo* efficacy and safety assays to identify drug candidates that have the potential of becoming preclinical & clinical candidates [4, 5]. In this chapter, we will describe a biopharmaceutics strategy to understand formulation issues and use this information to create formulation screens that can be used at the early stages of drug discovery research to de-risk drug candidates before entering preclinical development. The selections of appropriate formulations are based on a decision tree approach that is used by many pharmaceutical companies.

There are many excellent books and research papers that cover the theoretical [6–11] and practical [12–17] aspects of biopharmaceutics including drug formulation in the pharmaceutical industry or related industries. From a drug discovery biopharmaceutics point of view, physicochemical parameters of drug candidates including understanding the solid-state characteristics of the drug, solubility and permeability need to be evaluated with the highest degree of accuracy in the shortest amount of time using the least amount of drug compound (Fig. 1). Since the majority of drug candidates in drug discovery are solids at room temperatures, solid-state characteristics typically involve the investigation of salt type, polymorphism/amorphism tendencies, melting point, hygroscopicity, particle size distribution, specific surface area and stress stability. The characteristics of a drug candidate in solution involve dissolution rates, dissociation constants (Ksp), ionization potential (pKa), lipophilic partition coefficients (Log P) as a function of pH (Log D), hydrophobicity, and stability in solution. Molecular factors important to the permeability of a drug candidate include its molecular weight (MW), lipophilicity/hydrophobicity tendency, polar surface area (PSA) and the number of rotatable bonds (flexibility).

The relationship between solubility and permeability has been described by the Biopharmaceutical Classification System (BCS)

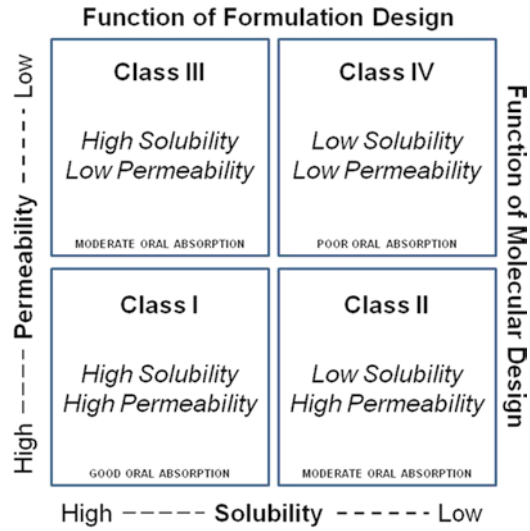

Fig. 2 Factors involved in oral absorption

classification (Fig. 2) [18, 19]. While the BCS was originally proposed to provide a scientific basis for an FDA biowaiver for conducting human bioavailability and bioequivalence studies, it is also a useful system to understand the formulation and molecular optimization needs of drug candidates [20, 21]. That is, oral absorption for small molecule drug candidates (i.e., compounds with MW <1,000 Da) is a dynamic process that involves the transfer of drug molecules from the stomach to the gastrointestinal lumen followed by transfer of the drug molecule across the apical intestinal epithelium membrane followed by diffusion through the cytoplasm and finally exiting through the basolateral membrane into the portal blood system (i.e., transcellular passage). The transcellular drug flux across the intestinal membrane is a product of the drug concentration (i.e., solubility) in the luminal fluid and the rate that the drug travels from the apical side of the epithelium cell to the basolateral side (i.e., permeability). Thus, a qualitative and quantitative understanding of solubility and permeability is essential in drug discovery to de-risk drug candidates against having poor oral absorption characteristics. Consider Fig. 2, a drug is classified as Class I if it has high solubility and high permeability; that is, good oral absorption characteristics. Hydrophobic drugs are typically classified as Class IV since they have low solubility and low permeability or, in other words, poor oral absorption characteristics. Class II and Class III represents drugs that have moderate oral absorption characteristics due to either having high or low solubility or permeability. Low and high cutoff values for solubility and permeability are typically based on animal or human physiological parameters.

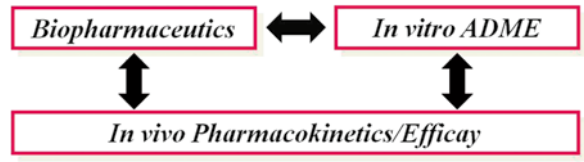

Fig. 3 Integrated "drug developability" assessment of drug candidates

By combining the biopharmaceutical profile as outlined in Figs. 1 and 2 with the results of early *in vitro* absorption, distribution, metabolism and excretion (eADME) assays [3, 4] leads to an integrated "drug developability" assessment of drug candidates for *in vivo* pharmacokinetic and efficacy studies (Fig. 3). Rapid and simple methods for classifying drug candidates according to Fig. 2 have been developed. However, a universally agreed upon choice of assays for solubility and permeability that provide an adequate biopharmaceutics assessment of drug candidates at the drug discovery stage has not been established. The primary reason for this situation is that drug candidate compounds, which are synthesized in small batches in drug discovery medicinal chemistry groups, are often only available in limited amounts (i.e., 5–20 mg) with varying degrees of purity (i.e., 70–95 %) from batch to batch. In addition, these candidate compounds may be available only as dimethyl sulfoxide (DMSO) stock solutions (i.e., 5–20 mM), which limit the range of assay options. As with all assays or combination of assays, the availability and purity of the drug candidate compound dictates the choice of assay used and the reliability of the data.

There have been more or less two approaches to biopharmaceutical profiling in a drug discovery environment: (a) using high-throughput screens (HTS) where drug candidate compounds are in DMSO stock solutions with varying degrees of purity and accuracy in stock concentrations; (b) using lower throughput screens where drug candidate compounds are initially solids with a higher degree of purity. Some pharmaceutical companies use one approach or the other while others use both approaches in a tiered strategy; that is, thousands of compounds are funneled through a series of high-throughput capacity solubility and permeability assays to lower capacity assays, which reveal more and more detailed information. A typical HTS approach to solubility might be the use of a kinetic or semi-equilibrium solubility assay [10, 22]. Here drug candidate compounds typically start as an inaccurate stock concentration DMSO solution that is both added directly to a buffer [23] or the DMSO is first evaporated and then buffer is added [24]. If the solubility measurement is taken in a short amount of time (i.e., 1 h), it is referred to as a kinetic measurement [25]; if the solution is allowed to equilibrate for a few hours to a day, the method is referred to as a semi-equilibrium measurement [24]. The buffer

used in these HTS approaches is typically a phosphate buffer at pH 6.5 or 7.4 [26]. The parallel artificial membrane assay (PAMPA) has been used as a HTS approach to permeability [27, 28]. In this type of assay, a lipophilic microfilter is impregnated with 10 % wt/vol egg lecithin dissolved in *n*-dodecane to create a filter-immobilized artificial membrane. The filter-immobilized artificial membrane is used in a chamber apparatus to separate an aqueous buffer solution containing the drug candidate compound at a known concentration from an aqueous buffer solution containing no compound. The kinetics of transport by diffusion across the artificial membrane is measured and a permeability parameter is calculated. While these HTS approaches to solubility and permeability provide approximate values, they are not accurate enough for conclusive biopharmaceutical profiling of drug candidates and many pharmaceutical companies have selected to stop using them. Balbach and Korn [29] have designed a series of lower throughput assays whereby the solid-state, and solubility characteristics of drug candidates can be evaluated in about 4-weeks using no more than 100 mg of highly purified drug compound. In their approach, the following solid-state characteristics of drug candidates are measured: the dissolution rates, dissociation constants (Ksp), ionization potential (pKa), lipophilic partition coefficients (Log P) as a function of pH (Log D), hydrophobicity, particle size distribution, polymorphism tendency, and stress stability at solid-state. The solubility and stability in pH 1.2–8.0 in fed-state simulating intestinal fluid (FeSSIF) and fasted-state simulating intestinal fluid (FaSSIF) are measured for each drug candidate. The human colorectal carcinoma intestinal cell line (Caco-2) is a cell culture model that is used to measure the permeability of drug candidates [30, 31]. Caco-2 cells spontaneously differentiate on microporous filter membranes into polarized monolayers with tight cellular junctions. The Caco-2 membrane is used in a chamber apparatus to separate an aqueous pH 6.4 buffer solution containing the drug candidate compound at a known concentration from an aqueous pH 7.4 buffer solution initially containing no compound. Drug molecule diffusion across the Caco-2 membrane, from the apical side to the basolateral side, with the permeability parameter being calculated based on the amount drug molecule reaching the basolateral side [32]. The Caco-2 cell model is designed to emulate transcellular drug flux across the intestinal membrane. The more prudent approach for an ideal biopharmaceutical profile of drug candidates would be some combination of moderate throughput solubility and permeability screens using the shortest amount of time (1–2 weeks), and the least amount of drug compounds (5–10 mg) with a high degree of purity (90–95 %).

Once the classification of a drug candidate has been determined, a formulation strategy for *in vitro* and *in vivo* assays can be established (Figs. 2 and 3). For example, in some cases, the solubility of a

drug can be improved, from low to high or from low to moderate, by optimizing its formulation. Thus, formulation strategies can be used to re-classify drugs; that is, change a Class II drug to a Class I drug. Formulation strategies for Class III or Class IV drugs may not improve their oral absorption characteristics since they are still compromised by their low membrane permeability. The best strategies for Class III and Class IV drugs are to make molecular design changes to improve their permeability based on physicochemical parameters outlined in Fig. 1. It should be understood that creating sufficient lipophilicity for membrane permeability and receptor binding, while polar enough for aqueous solubility, is not a trivial medicinal chemistry problem. Therefore, the correct choice of formulation of drug candidates in drug discovery is mandatory since many go/no-go decisions for the advancement of candidates are based on *in vitro* ADME and *in vivo* pharmacokinetic screens.

There are more or less three formulation approaches that are either used individually or in combination with each other to enhance the solubility of poorly soluble drug candidate compounds (i.e., <10 μg/mL) in a drug discovery environment [33–35]: (a) in some cases, the drug formulation strategy is oriented toward the creation of suspensions (i.e., supersaturating solutions) using polymers including methyl cellulose (MC), hydroxylethyl cellulose (HEC) or hydroxypropyl cellulose (HPC) [36]; (b) solubilization techniques using enhancers to aqueous media such as, buffers, cosolvents, surfactants (micellar system), and complexants are used to increase solubility [11]; (c) lipid-based formulations including lipid solutions, lipid emulsions, lipid dispersions, self-emulsifying drug delivery systems (SEDDS) and self-microemulsifying drug delivery systems (SMEDDS) have been investigated [37, 38]. In addition, rapid dissolving solid state formulations using drug particle engineering to enhance drug is also applied. These formulations include solid dispersions, nanoparticles and co-ground mixtures [39].

It is imperative that formulations need to be *in vitro* and *in vivo* biocompatible and stable. That is, the primary purpose of enhancing the solubility of poorly soluble drug candidates is to acquire sufficient *in vitro* and *in vivo* exposure without interfering with the experimental interpretation of the data. For example, using suspension formulations can lead to miss interpretation of the experimental results since the dissolution rate of the solid is typically not measured. In addition, physical stability of the drug candidate compound is a major issue of suspension formulations and short-term storage. Extreme pH conditions and cosolvents can have biocompatibility issues due to tissue irritation and drug precipitation in the lumen of the gastrointestinal tract. While cyclodextrins have an acceptable safety profile, there are still concerns of nephrotoxicity [40] especially at high acute concentration or in chronic studies. Some surfactants have systemic toxicity including histamine release and adverse cardiovascular effects and are poorly tolerated in chronic studies [34]. In general, nephrotoxicity is a

concern for lipid-based formulations. In addition, formulations should not mask the pharmacological effect being studied, such as, avoiding ethanol formulations when investigating CNS behavioral effects or dextrose-based formulation in diabetic animal models. While many formulations based on cosolvents, surfactants, and complexants will be acceptable for animal studies, they may not be acceptable in human studies.

We will focus on formulation approaches using buffers, cosolvents, surfactants (micellar system), and complexants that are either used individually or in combination with each other. Various solubilization techniques have been developed to alter the solubility and dissolution rates of small molecules in aqueous media [11–16]. These techniques range from simple methods such as the addition of 0.9 % sodium chloride (NaCl) or 5 % dextrose (D5W) to water for intravenous (i.v.) dosing to more complex strategies based on the addition of enhancers to aqueous media such as, buffers, cosolvents, surfactants (micellar system), complexants and lipids. A short list of common solubilizing enhancers is shown in Table 1 [33] where the recommended percent of the enhancer is given along with the route of administration of the dose. These solubilizing enhancers are either used individually or in combination with other enhancers or other solid-state particle size reduction techniques. For example, control of the pH of the aqueous media using buffers and particle size reduction using a mortar and pestle are the most common methods of enhancing the solubility of drug candidates that are weak acids or bases that ionize at physiological pH 2–9. Individual or combination formulations can be created using, cosolvents, surfactants, and complexants in combination with pH adjustments for weak electrolytes drug candidates along with solid-state nanoparticle size reduction methods [39].

Based upon these solubilization techniques (i.e., buffers, cosolvents, surfactants, and complexants) several formulation decision tree strategies have been reported in the literature. For example, Lee and coworkers [41] have applied these solubilization techniques to i.v. formulations for 317 drug candidates and were able to formulate over 80 % of the compounds. Gopinathan and coworkers [42] have applied these solubilization techniques to oral formulations for 26 drug candidates. Using 54 formulation conditions, all drug candidates could be formulated. The formulation conditions can be based on a decision tree approach using the pKa and Log P of the compound to guide the user in the selection of appropriate formulations (Fig. 4). Following the decision tree in Fig. 4, if the drug candidate molecule has acceptable aqueous solubility, then an aqueous formulation is selected. If the aqueous solubility is unacceptable and the drug candidate molecule has ionizable functional groups, a buffer formulation is attempted; if acceptable, a pH-based formulation is selected. If changing the pH is unacceptable and the Log P <3, then cosolvents are tried to improve the solubility. If this