Chapter 2
Discovery Formulations: Approaches and Practices in Early Preclinical Development

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2.1 Introduction

Technological innovations in biology, chemistry, and medicine have provided the pharmaceutical industry a wealth of targets and molecules with the potential to treat diseases once thought intractable to drug therapy. These advances have brought about a renaissance in the industry and current estimates suggest there are more than 5,000 potential new medicines in human testing, a high percentage of which would be considered "first in class" (Long and Works 2013). It has been suggested that pharmaceutical portfolios have shifted from commercially crowded therapeutic areas where the probability of approval is high to less crowded areas with novel targets and subsequent lower approval rates (Scannell et al. 2012). Additionally, there is a growing recognition that modulation of multiple targets (e.g., magic shotguns) rather than a single target (e.g., magic bullet) by a drug may provide greater therapeutic benefit to the patient (Roth et al. 2004; Morphy 2010; Gleeson et al. 2011). These transformations have resulted in a decline in new drug approvals and more importantly, a gradual but significant shift out of conventional druggable chemical space (Pammolli et al. 2011). The consequential increase in complexity, both in terms of the molecules and their biological targets, combined with the increasing need to work in an efficient and cost-constrained environment has necessitated an evolution in the role of pharmaceutical sciences in discovery support.

Traditionally, the pharmaceutical scientist participated on discovery teams only in the later phases of lead development or in the lead optimization phase, and their role was largely to assess the development risks (developability) of the molecule advancing to clinical dosing (Venkatesh and Lipper 2000). These activities, while

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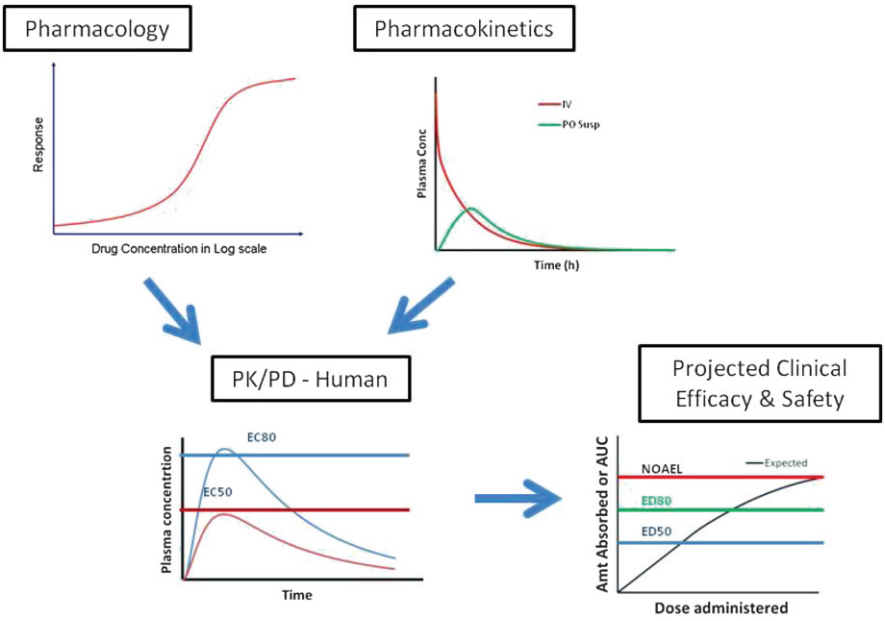

Fig. 2.1 Illustration of connectivity between pharmacology, pharmacokinetics, pharmacodynamics, toxicology and clinical dose range, and the relevance of discovery formulations and impact on clinical studies

important, have been augmented to include early discovery formulation support related to building a basic understanding of biology through in vivo target validation and demonstration of proof of mechanism (Neervannan 2006; Li and Zhao 2007; Shah and Agnihotri 2011). In addition, the desire to shorten development timelines while placing greater emphasis on patient centered design and delivery has brought about the need for development strategy discussions to start to take place earlier in preclinical development. Pharmaceutical scientists are ideally positioned to provide this type of support to project teams, given their knowledge of the physicochemical properties of compounds and training in formulation development (Hageman 2006). Formulations can profoundly impact drug release, absorption, and metabolism, which influence the resulting pharmacokinetic (PK) profile and the associated pharmacodynamic response. Thus, formulation and drug delivery technologies play an important role in in vivo discovery efforts.

The in vivo studies performed in the preclinical setting can broadly be classified as pharmacology, pharmacokinetic, and toxicology studies. The goals and challenges of these studies are diverse. Connectivity of key data collected from these studies, their impact on clinical formulation development, and ultimately on the in vivo clinical performance is illustrated in Fig. 2.1. The main output from pharmacology studies is the pharmacologic response (in the form of pharmacodynamic outcome, receptor occupancy, etc.) as it relates to in vivo plasma concentration or exposure of the compound. The primary outputs from a pharmacokinetic

Table 2.1 Preclinical formulations for in vivo studies in discovery: goals of studies and role of formulations

	Target to hit	Hit to lead	Lead optimization and clinical candidate selection
Pharmacology	Proof of concept/ target validation studies.	Compounds of interest from cell-based potency screens tested for in vivo activity. Multiple scaffolds not uncommon.	Nonoral routes of administration less common (if intent is oral dosing of clinical candidate).
		Wide range of concentrations to test for activity, target selectivity, and durability.	Studies focused on a thorough assessment of in vivo pharmacology for selection of clinical candidate.
		Time course and dose range assessments to understand on-target and off-target effects. Some studies done using nonoral formulations (e.g., IP or SC route).	
		Most formulation recommendation is based on assessment of selected compounds from each scaffold and vehicle effect considerations.	
	Mostly limited to 1 or 2 tool compounds, generally with poor druggability.	Formulations for expensive studies are based on compound-specific (or lot specific) assessments. Physical and chemical stability data generated as needed.	
	Single dose studies. Frequently high concentrations needed at the target.		
	Nonoral routes of dosing commonly used.		
PK/ADME	Limited to assessment of ADME properties of tool compounds in rodents	PK assessment in rodents to get basic understanding of clearance mechanisms and PK properties as they relate to scaffolds. Goal is to assist in selection of lead scaffold.	Rodent and nonrodent PK studies. Dogs are the most common nonrodent species.
			Oral absorption and metabolism parameters must be acceptable for oral dosing of clinical compound.
		Scaffold-wise formulation recommendation based on physicochemical properties of	Solubility and solid state data on material going

(continued)

Table 2.1 (continued)

	Target to hit	Hit to lead	Lead optimization and clinical candidate selection
		representative compounds. Basic crystallinity data on compounds of interest to inform formulation properties and/or absorption modeling.	into dog studies is essential. High emphasis on the absorbable dose in humans and potential need for enabled formulations.
			Formulation or study design options for overcoming PK variability associated with dog gastric pH may be used.
Toxicology	No in vivo studies	Short-term rat toxicology study on one or two compounds to support lead declaration. Doses up to 1,000 mg/kg not uncommon depending on potency data from pharmacology studies.	Short-term rat toxicology studies, followed by longer term or pilot toxicology studies to support clinical candidate selection.
			Rodent and nonrodent species.
Developability considerations	None	Preliminary assessment of developability to guide SAR.	Definitive assessment of commercial developability and understanding of associated risks.

study are the absorption, distribution, and clearance parameters for that compound as it relates to the species used in the study. These data feed into the generation of the pharmacokinetic–pharmacodynamic (PK-PD) model which describes the dose–concentration–effect relationship. Plasma exposures pertaining to the safety of the compound come from toxicology studies and provide a no observed adverse effect level (NOAEL), a level of exposure where there is no biologically significant increase in adverse effects compared to control. With appropriate scaling between species and the expected in vivo performance of the clinical formulation, the projected absorption and plasma exposures in the clinical dose range are established. Thus, while the formulations used for the various in vivo studies may be different, the outcomes of the studies are highly connected and have an important bearing on the design and execution of early clinical studies. Analysis of early clinical data enables further refinement of the models for next generation discovery efforts. In addition, the availability of exposure data from human studies allows for assessment of the performance of the drug product and provides a context for computational simulations of modified delivery systems, should the human pharmacokinetic profile suggest they are needed.

Table 2.1 summarizes the distinctive features and goals of preclinical in vivo studies based on the general type of the studies and the discovery phase during

which they are conducted. As noted in the table, formulations used in the early phases of discovery are geared toward target validation and/or proof of concept, with little or no developability considerations for the compounds or the formulations tested. As discovery programs progress toward lead declaration and subsequent optimization however, developability considerations take on increasing importance and the formulations used must be selected accordingly.

Strategies used in the development and assessment of preclinical formulations, and their application in the different types of in vivo studies will be discussed in the sections that follow.

2.2 Discovery and Preclinical Formulation Approaches

Formulation approaches to deliver molecules in the preclinical setting include suspensions, solutions, and amorphous dispersions administered as solids or in aqueous vehicles and each is discussed briefly below. These general approaches to formulation development, particularly related to solubilization, have been extensively reviewed and therefore, emphasis in this chapter is placed on application in the preclinical setting. The development of an overall formulation strategy to support in vivo studies should be considered carefully as it can reduce cycle time and resources. This strategy must be comprehensive, encompassing early studies designed to identify and validate drug targets, to long-term toxicology studies and ultimately, to support clinical studies in man. A focus on developing these types of strategies is presented in the next section, followed by a detailed discussion around practical considerations and examples for various types of studies, including pharmacology, ADME, toxicology, and alternate drug delivery.

2.2.1 Suspension Formulations and Nanosuspensions

Suspension formulations are the most widely used formulations in the discovery phase, owing to their ease of preparation and applicability to a wide variety of chemical platforms. In general, suspensions may serve as surrogates for exposure predictions from a standard human dosage form (capsule or tablet), provided the solid state properties of the compound are reflective of the API form to be developed. Standard suspension vehicles include a 1–10 % mixture in water of a cellulose polymer, such as methylcellulose, hydroxyethylcellulose, or acacia, accompanied by low levels (0.1–0.2 % w/v) of a nonionic surfactant such as polysorbate 80 to facilitate wetting. Use of nonionic components can minimize agglomeration due to charge interactions with ionizable drugs. Common practice in

the discovery setting is to reduce the particle size of the suspension using ultrasonic probe sonication, thereby creating more favorable properties for dissolution and absorption. With appropriate equipment configuration, a mean particle size diameter of 10 μm can routinely be achieved. One of the challenges with suspensions is that early lots of material often have less than ideal physical properties, which can include amorphous material or mixtures of amorphous and crystalline forms. This batch-to-batch variability can confound interpretation of in vivo results if consistent characterization of material in the dose preparation is not conducted. Additionally, the physical stability of the suspension must be monitored to ensure no form changes are occurring which may impact exposure. Daily preparation may help to avoid the need for this testing.

The use of nanoparticle formulations has much precedence in the discovery setting (Rabinow 2004). Nanoparticles are submicron (<1 μm) solid colloidal systems in which the drug is in a colloidal state of subdivision. This is in contrast to micronized drug where particles in the 2–5 μm range are typically achieved. Nanoparticles have a greater total surface area than the same mass of larger particles, resulting in increased dissolution rate. At very small particle sizes (e.g., <200 nm), saturation solubility may increase, however, the predicted increase is small, approximately 10–15 % at a particle size of 100 nm, according to the Freundlich–Ostwald equation (Kesisoglou et al. 2007). Nanoparticulate suspension formulations can be prepared on a small scale and in a short time frame using a variety of techniques (Merisko-Liversidge and Liversidge 2008) and can be administered by multiple routes (oral, intranasal, intraperitoneal, or intravenous). Key considerations in formulating nanoparticulate suspensions include appropriate choices of: (a) wetting agents for suspension formation and (b) polymers to coat the particles in order to create adequate steric hindrance for prevention of aggregation and particle growth. The nanomilling process should be optimized to achieve a very tight range for particle size distribution in the final product. This minimizes Oswald ripening and consequent particle growth in the suspension (Van Eerdenbrugh et al. 2008). Characterization should be performed in appropriate in vitro screens under biorelevant conditions, to ensure that the particles do not agglomerate upon dosing (Kesisoglou and Mitra 2012). The physical stability of the formulation should be monitored to ensure that no crystal form changes or undesired particle growth occurs during the intended shelf life. Formulating nanosuspensions for discovery is covered in Chap. 3.

2.2.2 pH Adjustment

Ionizable compounds with p*K*a's in the biorelevant range can often be formulated as pH adjusted solutions. The Henderson–Hasselbalch equation describes the relationship between the pH, p*K*a, and relative concentrations of the ionized and unionized forms of the compound in solution. The solubility of the ionized form is generally much greater than the solubility of the neutral form, and so the pH is

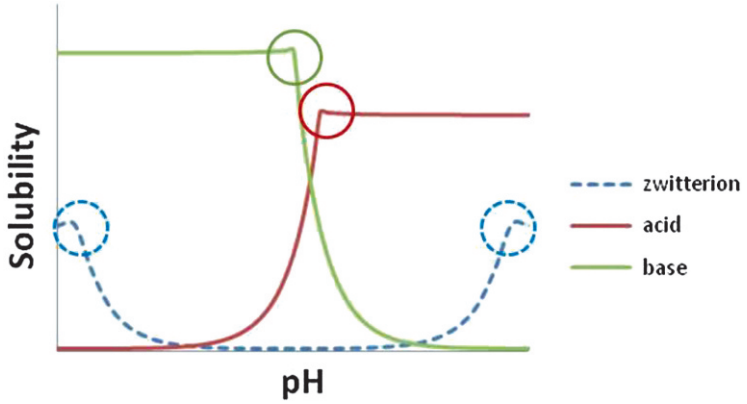

Fig. 2.2 pH–solubility relationships for different types of ionizable compounds. *Circles* indicate pH_{max}

modified in the direction of greater ionization as shown in Fig. 2.2 in order to achieve solubilization. Dilute solutions of sodium hydroxide or hydrochloric acid are most commonly used for pH adjustment. The final pH of the formulation should be monitored to ensure it is within an acceptable range for administration to laboratory animals, typically between pH 2 and 9 for oral administration. Chemical stability of the compound at the desired pH should also be verified. For compounds with high pH-dependent solubility in the pH range of the gut (pH 2–7), buffered systems may be used for short-term studies to minimize the risk of precipitation within the gastrointestinal tract. For parenterally administered formulations with a pH outside the range of approximately pH 6–8, it is important to make sure that the buffer capacity is sufficiently low (generally solution molarity should be ~25 mM or less) so as not to cause venous irritation.

Although pH adjustment increases the total amount of drug in solution, it should be recognized from the standpoint of oral absorption, it is the nonionized species that is more readily absorbed across the intestinal mucosa. In order to appreciate the effects of pH modification on solubility, it is necessary to consider the behavior of the various species present in equilibrium. The pH at which the ionized and unionized species are both saturated is referred to as the pH_{max} (Fig. 2.2). For basic compounds passing from the region of pH_{max} to regions of higher pH during GI transit, the equilibrium solubility at the corresponding pH could induce precipitation. However, depending on the intrinsic properties of the compound, or the composition of the local environment, it is fairly common for compounds to remain in a metastable state known as supersaturation for a significant duration of time. This increases the thermodynamic activity in the GI tract leading to enhanced flux and subsequent exposures (Pole 2008). However, supersaturation can also lead to precipitation of an acidic drug in the stomach, and a basic drug in the intestine, possibly leading to low and/or variable exposure. Precipitation can sometimes be

inhibited or reduced by the use of polymers added to the vehicle (Warren et al. 2010; Xu and Dai 2013).

Another effective method for obtaining a solution formulation via pH adjustment is the formation of an in situ salt, which is accomplished by adding molar equivalents of an acidic or basic counterion to the free form of an ionizable compound. This technique takes advantage of the different equilibrium constants (K_{sp}'s) that arise when different ions are present in a saturated solution of an ionic compound (Tong and Whitesell 1998). It is also useful when compounds prove difficult to formulate due to solid state issues encountered with the free form, such as poor suspendability or stickiness of the material in the formulation media. While the selection of an appropriate salt form is an important aspect of drug development, it is not usually practical to conduct a traditional salt screen in the early discovery phase. This challenge is readily overcome by the formation of an in situ salt. Typically, the p*K*a of the counterion selected should be approximately 2 p*K*a units away from that of the free form. For a basic compound, the counterion should have a p*K*a that is at least 2 units lower than the free form while for an acidic compound, the p*K*a should be at least 2 units higher than the free form. Due to the small amounts of material required, a number of different counterions can be screened rapidly in order to select the most desirable for use. It should be emphasized that since the primary goal of this approach is to find an aqueous formulation that offers the best solubility advantage, no consideration is given as to the long-term viability of the salt form identified.

2.2.3 Cyclodextrin Complexation

Cyclodextrins are cyclic sugar oligomers and their use in drug solubilization has been reviewed extensively (Stella and He 2008; Loftsson and Brewster 2010; Kurkov and Loftsson 2013). Cyclodextrins possess a hydrophilic exterior and a hydrophobic core, and therefore the primary mechanism of solubilization is due to the ability of these agents to form noncovalent inclusion complexes with lipophilic drugs. If the cyclodextrin–drug complex results from a 1:1 interaction, solubility increases linearly as a function of cyclodextrin concentration as shown in Fig. 2.3. The primary advantage this offers is low risk of drug precipitation upon dilution. Upon administration, dilution and competitive binding with plasma components are the major driving forces for dissociation of the complex (Stella et al. 1999; Kurkov et al. 2012). In most cases, dissociation is complete, providing rapid release of drug. However in a few cases, where the drug–cyclodextrin binding constant (K) is reported to be very high ($>1 \times 10^5$ M^{-1}), an effect on drug disposition has been observed (Charman et al. 2006). The importance of the binding constant has been detailed in an excellent review (Carrier et al. 2007) and these authors state that most poorly soluble drugs will have increased oral bioavailability when dosed as a cyclodextrin complex provided the binding constant is below 1×10^4 M^{-1}. Equally important is consideration of the drug and cyclodextrin concentrations in the

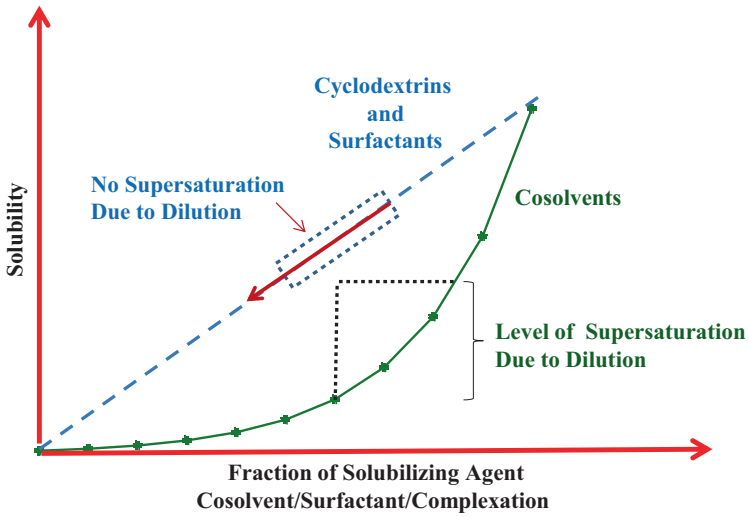

Fig. 2.3 Solubilization techniques—solubility vs. dilution. The dilution of cosolvent systems can result in supersaturation followed by precipitation. Cyclodextrins and surfactants typically increase solubility in a linear manner and therefore dilution does not result in supersaturation

formulation since these will influence the complexation equilibrium. In vivo performance may be impacted in cases where dilution is minimal (e.g., intranasal, intramuscular) or if concentrated, large volumes of cyclodextrins are administered orally (Stella and He 2008). This may be due to decreased uptake of the drug through biological barriers containing unstirred water layer (UWL) that exists between the membrane and bulk water (Loftsson and Brewster 2011) and a decrease in the free fraction available for permeability (Miller and Dahan 2012). The cyclodextrins most commonly used in discovery and development are 2-hydroxypropyl-β-cyclodextrin (HPβCD) and sulfobutylether-β-cyclodextrin (SBEβCD). These cyclodextrins are highly water soluble with solubility >500 mg/mL and have been extensively characterized with regard to safety profile and material properties. While both cyclodextrins are found in US marketed parenteral formulations, preclinical data suggests SBEβCD may be preferred for parenteral administration due to lower in vitro hemolysis compared with HPβCD (Shiotani et al. 1995; Luke et al. 2010).

Typical cyclodextrin use concentrations are approximately 10–20 % w/v. For example, the amount of cyclodextrin required for solubilization, given a target drug solubility of 10 mg/mL, a molecular weight of 500, and a 1:1 complex formulation, is 5 % w/v SBEβCD (average MW 2,163) or 2.8 % w/v HPβCD (average MW 1,400). Cyclodextrin complexation is often used along with complementary approaches such as pH adjustment and low levels of polymers to improve the extent of solubilization. In particular, SBEβCD carries a negative charge at physiological pHs due to the low p*K*a of the sulfonic acid groups. As a result, through charge attraction, cations may bind better than the neutral form to SBEβCD.

Thus, solubility screens include both neutral and ionized forms of the compound, where appropriate. In silico methods to predict binding constants, and therefore those compounds most likely to form complexes, are under development and may further guide compound selection in the future (Rao and Stella 2003).

2.2.4 Surfactants

Surfactants are amphiphilic molecules containing both hydrophilic and hydrophobic regions. In aqueous solutions at concentrations above a critical micelle concentration (CMC) they form aggregates, such as micelles, where the hydrophilic region is oriented to the bulk media and the hydrophobic region is oriented toward the core. They can be useful additives when hydrophobicity of compounds is the limiting factor for solvation in aqueous media, or when the molecule itself is amphiphilic. Solubility of compounds that are amenable to this approach generally increases in a linear manner with increasing surfactant concentration. As a result, the risk of precipitation upon dilution of surfactant-based formulations is minimal as depicted in Fig. 2.3. Several authors have published lists of common surfactants and typical formulation concentrations for both oral and IV use (Neervannan 2006; Li and Zhao 2007; Strickley 2008). Examples of commonly used surfactants include polysorbates (e.g., Tween 80) and polyoxyl castor oil (Cremophor EL). In general, the primary challenge with the use of surfactants in preclinical formulations is the large amounts of these excipients typically required for solubilization, which are associated with tolerability issues. Hypersensitivity reactions have been well documented following intravenous administration of certain surfactants such as Cremophor EL and polysorbate 80 to sensitive animals (particularly dogs) and humans (Lorenz et al. 1977; 1982; Weiss et al. 1990; ten Tije et al. 2003). Changes in plasma clearance with the use of surfactants have been reported both in vitro and in vivo (ten Tije et al. 2003; Bravo et al. 2004; Bittner et al. 2005). Inhibitory effects on intestinal drug transport processes such as P-glycoprotein (P-gp) efflux are well established for numerous surfactants and details have been compiled in a review (Williams et al. 2013). Thus, care must be taken in interpretation of pharmacokinetic and pharmacodynamic data generated in the presence of high concentrations of surfactants. The use of Vitamin E-TPGS to increase the bioavailability of poorly soluble drugs is well document (Li et al. 2012). However, oral administration of Vitamin E-TPGS in longer term studies may result in absorption of D-α-Tocopheryl (Vitamin E) by the hydrolysis of the TPGS moiety (Traber et al. 1986; Dimitrov et al. 1996; Jacquemin et al. 2009). This can lead to exposures to Vitamin E that potentially could complicate interpretation of pharmacology or safety studies. Therefore, the nature of the surfactant and level of use should be carefully considered in designing formulations.

2.2.5 Cosolvents

Organic cosolvent systems can be powerful solubilizing agents for molecules in the discovery setting. Cosolvents alter the polarity of aqueous systems to provide a more favorable solubilization environment for nonpolar solutes. Most cosolvents are characterized by hydrogen bond donor and acceptor groups that interact strongly with water and help ensure mutual miscibility in practically any proportion. They also have small hydrocarbon regions that do not interact strongly with water. These hydrocarbon regions reduce the ability of the aqueous system to squeeze out nonpolar solutes. As a result, cosolvency is a highly versatile and powerful means of solubilizing nonpolar solutes in aqueous media (Yalkowsky 1999). Typical cosolvents include *N*-methyl pyrrolidone, 2-pyrrolidone, dimethylsulfoxide, polyethylene glycol 400, and dimethylacetamide. The solubility profile as indicated in Fig. 2.3 follows a log-linear relationship with cosolvent concentration. Therefore, cosolvent-based formulations, if formulated near their solubility maximum, will supersaturate upon dilution during parenteral or oral administration. With supersaturation comes the possibility of precipitation in the in vivo environment causing low or variable exposure. Orally, this may sometimes be mitigated using small amounts of polymers or surfactants (Gao et al. 2004; Xu and Dai 2013). Toxicity and tolerability of cosolvents should be a consideration and often limit their use for studies with high dose requirements (necessitating larger volumes of cosolvents to be dosed) or studies with long duration (such as toxicology testing). When used in parenteral formulations, hemolysis may occur, which can cause pain due to the release of hemoglobin from erythrocytes into the plasma. An in vitro screening approach, as described by Reed and Yalkowsky (1985) may be used to evaluate the hemolytic potential of formulations. Additionally, cosolvents (particularly PEG400) have been shown to interfere in mass spectrometry based bio-analytical methods due to ion suppression, in which the analytical response for the compound of interest is reduced due to the coelution of an excipient (Larger, Breda et al. 2005). Once identified as an issue, this problem can generally be overcome, either by altering the HPLC method or by reducing or eliminating the excipient responsible for interference.

2.2.6 Lipids

Lipid-based formulations can be an attractive formulation approach for molecules with high $\log P$ (>4). These formulations can include simple oils to emulsions, microemulsions, self-emulsifying and self micro-emulsifying drug delivery systems (SEDDS/SMEDDS). SEDDS and SMEDDS are mixtures of lipids, surfactants, and cosolvents that disperse in aqueous media to form emulsions or microemulsions and have been effectively used to increase exposure of highly lipophilic molecules and been reviewed extensively in the literature (Porter

et al. 2008; Pouton and Porter 2008; Williams et al. 2013). The in vivo performance of these formulations depends on how they are processed in the gastrointestinal tract. For example, formulations composed of long chain triglycerides undergo lipolysis and the digestion products are further solubilized by bile salt–lecithin complexes, resulting in the formation of fine colloidal dispersions that bypass first-pass metabolism and are predominantly absorbed through the intestinal lymphatic system. Thus, bioavailability of compounds formulated in this manner can be greater than what might be achieved when solubilized compound is absorbed through the standard mechanism via the portal system. For example, lipid-based formulations make it possible to effectively deliver testosterone derivatives via the oral route by targeting the lymphatic system and reducing first pass liver exposure (Dudley 2011; Yin et al. 2012). In order for compounds to be amenable to these formulations, their solubility in the lipid system should be sufficiently high to support the dose requirements for animal studies. This is often a limitation to the use of this formulation approach. Additionally, chemical and physical stability of the compound in the vehicle/dosage form can sometimes be a major hurdle for long-term use and should be studied carefully (Pouton and Porter 2008). The impact of lipid-based components on the clinical pharmacological parameters being assessed in the study, and their safety and tolerability also need to be assessed as they can significantly restrict the amounts used and the duration of the studies. Despite the barriers, it is possible to leverage the numerous advantages offered by lipid-based formulations. A recent review by Chen et al. describes an effective strategy for incorporating lipid-based formulations into discovery flow schemes, such that the properties of the chemistry templates can be appropriately influenced in order to make them viable candidates for lipid-based formulations. This is especially valuable when the intrinsic properties of the biological targets do not lend themselves to ligands that can be delivered through conventional formulation approaches (Chen et al. 2012).

2.2.7 Solid Dispersions and Supersaturation

Amorphous solid dispersions are enabled oral formulations that have received a great deal of attention in the discovery phase. This is primarily due to the observed increases in exposure in animal and human testing, the small scale in which solid dispersions can be manufactured, and relative safety of the excipients used. Amorphous solid dispersion formulations are dispersions of amorphous drug in a carrier matrix (usually a polymer). They form supersaturated solutions upon dosing, thereby increasing the flux across the intestinal membrane. With appropriate choice of the polymer, it is often possible to sustain the duration of supersaturation for several hours, thereby overcoming absorption limitations due to low equilibrium solubility. Polymers and other excipients used to make amorphous solid dispersions generally have greater acceptable daily intake (ADI) amounts compared to the excipients used to make the simpler formulations described in the previous

paragraphs, which makes solid dispersions an attractive option for longer term studies. A number of innovative products have reached the market in recent years which have been developed as solid dispersions in order to overcome solubility limitations of the crystalline forms of the drugs that were found to negatively impact the performance of the drug product (Vo et al. 2013). The use of amorphous solid dispersion formulations in discovery has also been described (Verreck et al. 2003; Vasconcelos et al. 2007; Bikiaris 2011). In our experience at Lilly, amorphous solid dispersions have been successfully applied for oral dosing in both toxicology and clinical studies and have resulted in significant improvements in plasma exposure and decreases in variability compared to conventional formulations with crystalline material.

Solid dispersions present a greater level of complexity when compared to the other formulation approaches that have been described. They require greater resources for formulation development and preparation of supplies for in vivo studies. In addition, the chemical and physical stability of the solid dispersion formulation must be carefully evaluated to ensure that it possesses sufficient handling and storage characteristics for use in the desired study. Briefly, the development process includes small-scale experiments to select the drug–polymer combination that results in the best dissolution profile, followed by a slightly larger, but still milligram scale set of experiments, to assess thermal properties and physical/chemical stability of the formulation (Six et al. 2004; Vandecruys et al. 2007; Qian et al. 2010). Owing to the nature of the manufacturing processes for amorphous solid dispersions, adequate overages need to be built in to material estimates to cover for loss during production and handling. This option is therefore utilized only for compounds that do not lend themselves to any other options, or when the specific needs of the in vivo studies preclude the use of excipients that would be required with the other available formulation approaches.

2.3 Formulation Strategy

The previous section describes *Formulation Approaches* for drug solubilization using various techniques (aqueous and cosolvent) or the use of particulate/solid dispersion systems. The question remains as to a viable *Formulation Strategy* to determine which approach is best suited to the molecule of interest. A number of recent publications have presented flow charts or high throughput screening paradigms as a means to "zero in" on formulations most amenable to the compound being tested (Chaubal 2004; Li and Zhao 2007; Maas et al. 2007; Saxena et al. 2009; Balazs 2011). These flow charts tend to be a linear progression of various in vitro assays, evaluating a broad range of excipient classes and solubilization methods. Logical in design, these approaches ultimately require an iterative process of in vivo testing and reformulation in order to identify a formulation capable of producing the desired exposures. This may not be conducive to the

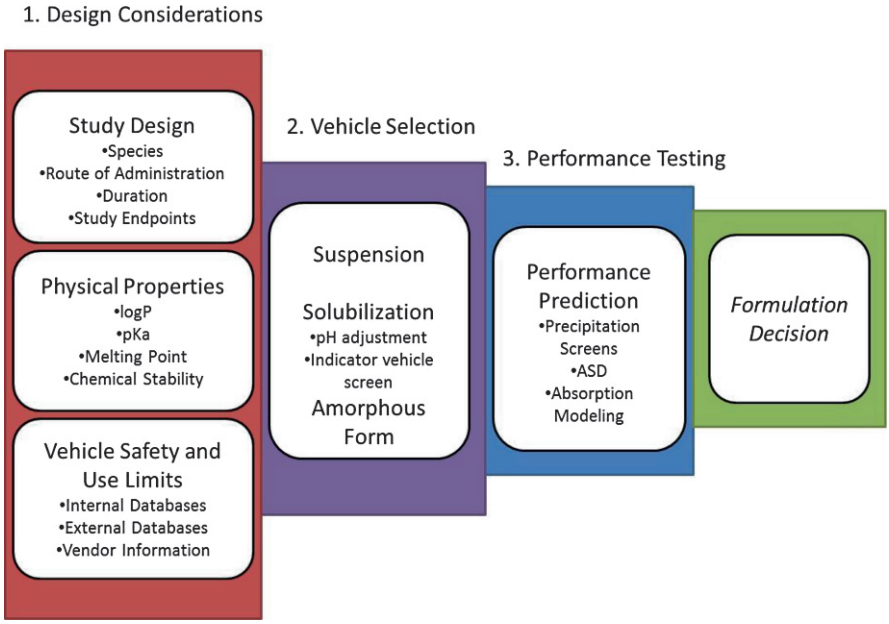

Fig. 2.4 Streamlined paradigm for the selection and evaluation of preclinical formulations

speed required in the discovery phase because iterations can be time consuming and require relatively large quantities of drug.

An alternative strategy for identifying preclinical formulations in a more streamlined manner is outlined in Fig. 2.4. This strategy relies on three integrated steps, each with its own unique set of models and tools. The first step consists of an assessment of the physicochemical properties of the compound combined with a careful evaluation of the in vivo study parameters. The physicochemical properties for the compound of interest are obtained through in vitro measurement (e.g., solubility, pH stability, permeability) or through the use of in silico models. This allows a molecule to be described by a few fundamental properties that can be tied to potential methods of solubilization, such as ionization potential (p*K*a) and lipophilicity ($\log P$). This must then be considered within the context of requirements for the in vivo study such as species, dose, route of administration, duration, etc. Based on these data, an initial list of preferred vehicles is generated. The list of vehicles is further narrowed by incorporation of important excipient data related to safety and potential pharmacokinetic/pharmacologic interference. These data are derived from a number of different sources, including external data from the literature, vendors, and from compilations of extensive internal in vivo study data. At the completion of this evaluation, an initial hypothesis is generated as to the general types of vehicles that would likely be successful in meeting the requirements of the study as well as what methods of solubilization would best take advantage of the inherent structural properties of the molecule. In doing so, the relatively exhaustive list of possible excipients can be narrowed simply by

eliminating those vehicles that are not compatible with the functional groups present in the compound or the design of the in vivo study.

The next step in this strategy centers on the use of in vitro screening tools to determine if solubility targets are achieved. As shown in the figure, vehicles are subdivided into three broad categories: suspensions, solubilized formulations, and stabilized amorphous formulations. From the analysis conducted previously, one or more of these classes would have been identified as an appropriate starting point for formulation development, based on the type of compound and study. Studies where only a single dose is to be administered, such as a pharmacokinetic study, we have found that an appropriate vehicle can be selected from a defined list of "standard" vehicles, which has been developed using institutional knowledge. These vehicles, as well as recommended characterization to facilitate interpretation of in vivo data are summarized in Table 2.2. Rather than screening all possible excipients and

Table 2.2 Standard vehicles for use in single dose studies

Route	Formulation	Minimum characterization
Oral suspensions	•HEC 1 % w/v with PS80 0.25 % v/v and simethicone 0.05 % v/v •Acacia 10 % w/v with simethicone 0.05 % v/v	Visual, microscopy
Oral enabled formulations	•Cyclodextrin (20 % w/v) with or without pH adjustment	Microscopy of solid forms
	•PEG 600 90 % v/v, Solutol® HS 15 10 % v/v	
	•Soybean Oil 80 % v/v, Capmul® PG8 20 % v/v (if clogP > 5)	
	•Gelucire® 44/14 100 %	
	•Solid dispersion (30–50 % w/w polymer)	
	•Nanosuspension (PVP 2 % w/v and SLS 0.15 % w/v for steric stabilization and wetting)	
PK Intravenous dosing (single dose only, 1 mL/kg)	•SBEβCD 20 % w/v, 25 mM pH 2 or 8 $NaPO_4$ buffer	In vitro plasma precipitation screen (if cosolvent)
	•Microemulsion 20 % water	
	•DMA 10 % v/v, EtOH 15 % v/v, PG 30 % v/v in 25 mM pH 2 or 8 $NaPO_4$ buffer	
	•DMA 25 % v/v, EtOH 15 % v/v, PG 10 % v/v, 2-pyrrolidone 25 % v/v (last resort!)	
Intraperitoneal/subcutaneous dosing	•SBEβCD 20 % w/v in 25 mM pH 3 or pH 8 $NaPO_4$ buffer	Visual
	•NMP 10 % v/v, Captex 300 or soybean oil 90 % v/v (Last resort!)	

Solutions/suspensions prepared using deionized water
HEC = hydroxyethyl cellulose, PS80 = polysorbate 80, PEG = polyethylene glycol, PVP = polyvinylpyrrolidone, DMA = dimethylacetamide, NMP = *N*-methylpyrrolidone, PG = propylene glycol

combinations thereof, a vehicle is selected from this abbreviated list which contains vehicles for each route of administration that we have found have the highest success rate for the types of study. In cases where solubilization has been proposed as a means to achieve the desired exposure target, we have devised a means of screening a broad range of solubilization methods using a very limited set of representative vehicles as opposed to conducting solubility determinations in large numbers of individual excipients. If the results point to intractable solubility space where it would be necessary to use vehicle(s) not amenable to the study design (e.g., aggressive cosolvents in long-term toxicology testing) then alternate technologies such as solid dispersions or adjustment in dose regimen are considered.

As will be discussed later in this chapter, toxicology studies are somewhat unique from most other in vivo studies conducted in the discovery setting. The need to achieve high exposures, either by administering large doses of compound or through the use of enabling formulations, can make the identification of a suitable vehicle challenging. For short investigative toxicology studies, a relatively wide range of vehicles may be used, as excipient toxicity should be minimized due to the limited duration. However, from the standpoint of formulation development, toxicology studies conducted late in the discovery framework are primarily designed to identify acceptable vehicles for use in longer duration FHD-enabling studies in early clinical development. As a result, greater consideration must be given to both the safety profile of the excipients used in these formulations as well as the complexity of manufacturing and formulation stability that will be required.

Prediction of the potential in vivo performance of formulations makes up the final step in designing and implementing an efficient formulation strategy. This is of particular utility when there are several viable formulation options to choose from or when considering high dose administration typically encountered in toxicology studies. As discussed previously, the use of in vitro systems is preferred as they allow for evaluation of formulations without the need for large numbers of costly and time-consuming in vivo studies. A particularly useful tool for this purpose is the Artificial Stomach Duodenum model (ASD) (Carino et al. 2006), which is a dynamic dissolution system that simulates the pH and mass transfer of the stomach and duodenal compartments. By comparing of the duodenal dissolution profiles of various test formulations, the relative supersaturation of each may be evaluated, which theoretically correlates with the rank order of absorption of compounds displaying solubility limited absorption. This type of system has been further simplified by Gao and coworkers (2010) who described a pH-dilution method which mimics the relevant pH, volumes, and transit times in the gastrointestinal tract of the rat. Much like the ASD, this simple method has been used to estimate regional changes in drug concentration along the GI tract for various formulations. Results from these types of in vitro systems are often used in conjunction with absorption modeling, which will be discussed in more detail later in this chapter. Absorption modeling is used to predict the relative in vivo performance of formulations by simulating plasma exposure profiles, which makes it possible to explore many different hypotheses simply by modifying the simulation parameters.

In the discovery phase, the formulator is often asked to develop formulations that would be generally acceptable for any compound within a given scaffold of interest. This enables compounds to be progressed rapidly through in vivo assays without incurring delays associated with developing novel formulations for each compound one at a time. As has been outlined in the aforementioned strategy, scaffold-wise formulation recommendations must also be based on careful assessment of an adequate number of compounds, and a proper understanding of the relationship between specific structural motifs, compound properties, and the underlying approach to the formulation. Above all, it is essential that there is a well-defined feedback loop within the discovery team, so the preformulation/developability scientist is aware of the performance of the formulations, and any unusual observations with regard to the physical appearance of the formulations, or the in vivo response and/or exposure.

While many different approaches to formulation strategy may exist across the pharmaceutical industry, they share a common goal of working to identify formulations that support the progression of new molecules through the discovery pipeline in a rapid and efficient manner. Given the highly complex nature of the process of drug discovery, it is clear that formulation development plays a significant role in the overall success or attrition of discovery projects.

In the sections that follow, additional discussion and examples of formulation development for various types of studies are presented.

2.4 Pharmacology Formulations

It should be appreciated that preclinical formulations strongly influence the link between pharmacology, pharmacokinetics, and pharmacodynamics. The biological targets being explored today are far more complex than those of a decade ago (Hopkins and Groom 2002), and the cost of typical pharmacology studies can exceed $40,000–50,000 due to the highly sophisticated nature of the design (e.g., in vivo efficacy studies in xenograft models) as well as long lead times due to study preparation. Finally, as a result of the recent emphasis on translational research and the development of biomarkers, there is a much greater focus on the identification of both outcome and mechanism biomarkers early in discovery (Kwong et al. 2011). It is therefore critical that the formulations used in pharmacology studies are designed to perform consistently and reproducibly in order to meet the needs of these studies and to drive the right decisions.

Formulation needs for pharmacology studies gradually shift over the different phases of discovery efforts as presented in Table 2.1. In the early phase of most projects, the primary goal is to test a biological hypothesis for a mechanism of action or to validate a novel biological target. Many experiments are run with compounds that have not yet been optimized and therefore, have poor druggability properties, including incomplete absorption upon oral dosing and/or rapid clearance. In addition, there is rarely enough information available at this stage

Table 2.3 Summary of the various routes of administration used in in vivo studies

Route of administration	Typical reasons for choice	Comments
IV (bolus or infusion)	1. Maximize exposure by avoiding first-pass metabolism, e.g., cell cycle targets in oncology and in acute invasive studies in metabolic disorders.	Solubility could be a limiting factor for amount that can be delivered through this route.
	2. To overcome oral absorption limitations such as permeability and first-pass metabolism.	
	3. IV is intended route in clinical development.	Injection site irritation potential may also limit the use of this route (Turner et al. 2011).
	4. Infusions are used to achieve sustained exposures to assess PD response at longer time points.	
SC	1. Similar to IV, but this route is also amenable to suspension formulations for bolus dosing. This is useful when solubility limitations preclude use of the IV route (neurodegenerative, behavioral pharmacology, diabetes targets).	Dose volume, solubility (for solution-based formulations), or suspendability for (suspensions) may be limiting factors.
	2. To overcome oral absorption limitations such as permeability and first-pass metabolism.	Injection site irritation may limit the use of this route.
	3. Use of osmotic pumps for sustained target engagement, especially with rapidly cleared compounds.	In addition, in pain studies, e.g., pain due to injection can obscure the efficacy of the compound.
IP	1. Compounds with absorption limitations due to low permeability across intestinal mucosa.	Compounds will enter the portal vein immediately upon dosing and be subject to first-pass extraction similar to oral route (Lukas et al. 1971). The ability of the lymphatic system to drain the peritoneal cavity may be important in the absorption of proteins and large molecular weight compounds (Mactier et al. 1987)
	2. Mechanism of action studies (e.g. intraperitoneal glucose tolerance test in diabetes) to assess PD outcome independent of incretin response in the gut.	
	3. To avoid stress of oral dosing in some behavioral pharmacology studies.	

(continued)

Table 2.3 (continued)

Route of administration	Typical reasons for choice	Comments
IC/ICV/IT (IC: intracerebral; ICV: intracerebroventricular; IT: intrathecal)	1. To deliver high local concentrations of drug directly to CNS or specific tissues in the brain, to demonstrate/understand mechanism of action (pain pharmacology, neurodegenerative, and behavioral pharmacology).	Solubility could be a limiting factor, especially for compounds that will require high doses in order to saturate P-gp efflux.
	2. To overcome P-gp efflux that inhibits compounds crossing the blood–brain barrier from plasma.	
PO—oral gavage	1. Most common route. Applied across target-to-hit, hit-to-lead, and lead-optimization phases.	Gastric pH variability (in dog) can result in significant PK variability for basic compounds. Can be overcome by formulating the compound as in situ salts or in acidic media with sufficient buffer capacity.
	2. Essential route to bridge to efficacious exposures in humans for orally administered drugs.	
PO—in feed dosing (Mu et al. 2006), or in water	Convenience, sustained plasma concentration, large doses, combination therapies, or to induce disease states through drugs.	Taste factors and feeding patterns can affect intake. Food wafers have been reported to be useful (Ferguson and Boctor 2009). Modeling approaches may be used to guide amount incorporated into the water or feed.

regarding the duration of target engagement needed to elicit the desired pharmacologic response. As a result, formulations used in this phase of work are designed to provide maximum and/or sustained exposure and often use nonstandard routes of delivery to help further explore these concepts. Table 2.3 lists the various routes of administration used in in vivo studies and considerations for selecting one over another. Table 2.4 summarizes examples of vehicles and typical dose volumes for the preclinical species commonly used in pharmacology studies. In general, for projects that are focused on delivering clinical candidates for oral administration, pharmacology studies utilizing nonoral routes should be considered nonstandard and the exposure data obtained should not be used in making assessments of the potential for oral absorption.

The approach to identify formulations for these studies utilizes the strategy previously described, taking into account intended goal of the study, the need for single vs. chronic dosing, safety of formulation excipients, and any vehicle or dosing effects on the pharmacologic response or clinical parameters secondary to the pharmacologic response. The Lhasa Vitic Nexus database contains an exhaustive and continuously updated repository of information on excipient safety in preclinical species. Given that chronic dosing is often required in animal

Table 2.4 Summary of vehicles example and typical dose volumes for the preclinical species commonly used in pharmacology studies

Route	Species and dose volume (mL/kg)	Examples of vehicles	General comments on formulation
IV	Rat: 1 mL/kg (≤5 mL/kg recommended max) Mouse: ≤5 mL/kg Dog: 1 mL/kg (≤2.5 mL/kg may be acceptable if needed)	1. Deionized water with pH adjustment for solubility (acceptable pH range 2–8), or 25 mM buffers, pH 2 or pH 8. 2. ≤20 % cyclodextrin (e.g., SBEβCD, HPβCD), in 25 mM pH 2 or pH 8 buffer, and/or with pH adjustment in the range of 2–8. 3. Cosolvent based (e.g., 10 % DMA, 15 % EtOH, 30 % propylene glycol in pH 2 or pH 8 buffer).	Minimum volumes and lowest acceptable dose must be used to avoid formulation failure. Risk of precipitation upon injection must be tested using a precipitation screen.
SC	Mouse: 10 mL/kg Rat: 1 mL/ recommended (≤10 mL/kg may be acceptable) Dog: 1 mL/kg recommended (2 mL/kg may be acceptable)	Normal saline with small amounts of suspending and/or wetting agents (e.g., 10 % Cremophor EL, 1 % Hydroxypropylcellulose, 0.085 % Polyoxyl-50-stearate). Cosolvent based formulations may be used for osmotic pump infusions (e.g., 1:1 PEG400: DMSO).	Vehicles must be tested for irritation potential, especially for repeat dose studies. Osmotic pump formulations must use only excipients compatible with pump components. Formulations must be tested for delivery and precipitation potential.
PO	Mouse, rat: 10 mL/kg Dog: 5 mL/kg Monkey: 3 mL/kg	Solution: •DI water with pH adjustment for solubility (acceptable pH range 2–8), or 25 mM buffers, pH 2 or pH 8. •≤20 % Captisol or HPBCD with pH adjusted to 2 or 8. 0.5 M phosphoric acid, pH 2. Suspensions: •1 % hydroxyethylcellulose, 0.25 % polysorbate 80, 0.05 % Antifoam in DI water. •10 % acacia, 0.05 % antifoam in DI water.	*Examples of vehicle effects*: Cremophor EL, Triton X-100, Polysorbate 80, Solutol HS15, PEG400 have been known to alter plasma lipoproteins, resulting in significant interference with metabolic disorder studies. The mild anti-inflammatory effect of acacia has been known to impact arthritis models when used in suspension vehicles.
IP	Mouse: 20 mL/kg Rat: 10 mL/kg	Solution or suspension formulations	Solubility of the compound can be a limiting factor for absorption when dosed as a suspension.
IC/IV/IT	Rat: ≤10 μL Mouse: ≤5 μL	Normal saline, phosphate buffered saline, artificial CSF	

pharmacology models, this data is useful in selecting formulations that have sufficient safety for the proposed study duration. Several reviews on preclinical formulation and related topics describe examples (ten Tije et al. 2003; Gad et al. 2006; Neervannan 2006; Li and Zhao 2007; Maas et al. 2007; Pole 2008; Shah and Agnihotri 2011) of vehicle effects that interfere with pharmacology studies. Acacia (a commonly used suspending agent) interferes with pain and inflammation models (Lilly internal experience and (Dafallah and al-Mustafa 1996)), and therefore should generally be avoided for these types of studies. Figures 2.5 and 2.6 illustrate vehicle effects from Lilly internal experience. Figure 2.5 shows inflammation clinical scores upon dosing 10 % acacia and 1 % hydroxyethylcellulose as aqueous dispersions at a dose of 4 mL/kg. These are commonly used suspension vehicles, but as seen from the figure, the 10 % acacia vehicle has a positive anti-inflammatory effect that precludes the use of this vehicle in this pharmacology model. Figure 2.6 shows the effect of some standard formulation excipients on insulin release in the Oral Glucose Tolerance Test in mouse. As indicated in the figure, PEG400 has a statistically significant negative effect on insulin release that makes it unacceptable for use in this model. Another example of vehicle effects found in the literature is that of Cremophor EL (an emulsifier used in parenteral formulations), which affects plasma lipoproteins when used at concentrations greater than 0.4 mg/mL (Woodburn and Kessel 1994). These examples illustrate the importance of checking for vehicle effects either through experimentation or literature examples prior to making decisions for pharmacology studies.

2.4.1 Osmotic Pumps

The preceding paragraphs describe aspects of formulations and various routes of administration in animal models. However, overcoming poor exposures resulting from rapid clearance poses a unique challenge in pharmacology studies. Furthermore, there may be a need to maintain sustained plasma concentrations over several hours to gain useful mechanistic insights in some pharmacology models. Typical examples are kinase inhibitors where sustained target engagement is essential to block the signaling pathways responsible for cancer cell growth. This requirement, coupled with rapid clearance, is a fairly typical challenge faced during the target validation phase of many discovery projects. The simplest way to address clearance issues is to dose the compound multiple times a day. However, this is not always practical. For example, the increased handling of animals for administering multiple daily doses can cause stress that could confound the PD response in neuroscience studies (Gartner et al. 1980). Gastric-retained gel formulations have been used with some success to modulate pharmacokinetic profiles of rapidly cleared drugs (Foster et al. 2013) but in vivo performance is somewhat difficult to predict based on in vitro assessment.

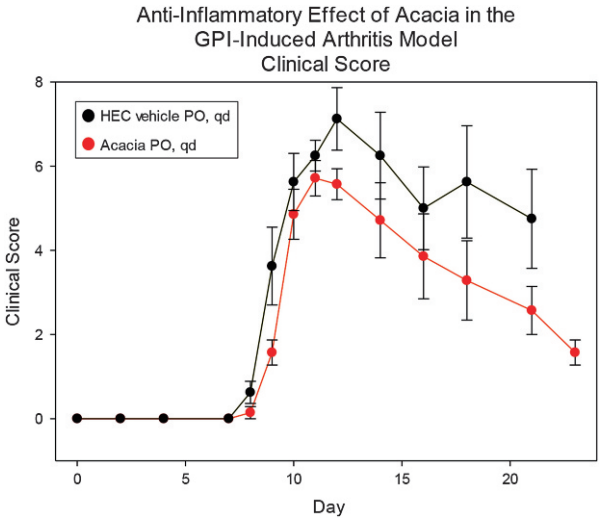

Fig. 2.5 Illustration of the effect of acacia on GPI-induced arthritis model in mouse

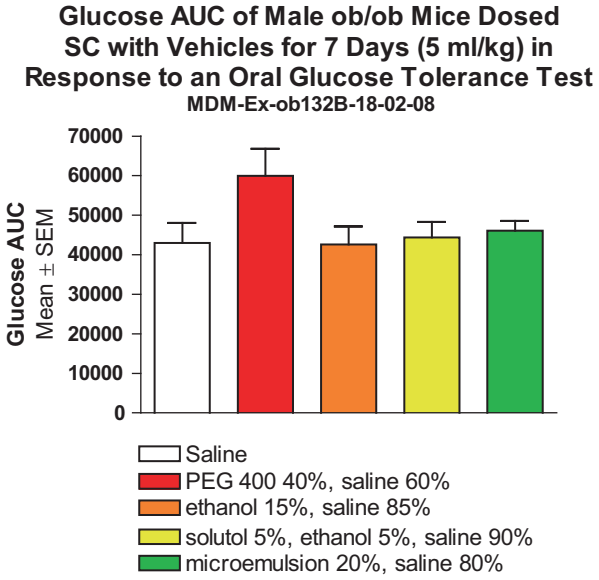

Fig. 2.6 Illustration of the effect of PEG400 on insulin response in the mouse OGTT model

One of the more widely used approaches to maintain sustained plasma profiles for extended durations of time is through infusion of the drug through parenteral routes. This is commonly achieved through the use of surgically implanted osmotic pumps. Osmotic pumps deliver at a constant rate and can be used to maintain a nearly constant plasma concentration and thus continuous target engagement for up to 2 weeks. In contrast to conventional dosing options that result in large peak to

trough ratios, osmotic pumps also offer the secondary benefit of minimizing the total dose by eliminating the portion of the AUC in the plasma concentration profiles that is above the threshold for activity/efficacy. An excellent example of this was reported by Kumar et al. who reported on comparative in vivo efficacy responses upon dosing orally and via osmotic pumps implanted subcutaneously. Efficacy was assessed as a reduction in tumor volume and decrease in hemoglobin in the biopsy tissue (the latter is a measure of antiangiogenesis). As seen from their work, the doses required for efficacy were significantly lower when the drug was delivered via the SC osmotic pump as compared with oral (Kumar et al. 2007). These pumps are available in a wide range of capacities and delivery rates to suit the various preclinical animal models that are used in pharmacology studies (Alzet). Newer pump models such as those from Iprecio (Iprecio) are programmable for variable flow rate if needed, and re-fillable, thus enabling larger doses and/or longer duration studies. Formulation technologies that provide extended release are described in Chap. 3.

The first step in developing a formulation for osmotic pump studies is to select an appropriate pump model based on the animal species being used and the duration of the study. Based on the capacity of the pump, the volumetric delivery rate specific to the selected model, and the desired plasma concentration, the required formulation concentration is then estimated using the following equation with appropriate unit conversions:

$$\text{formulation concentration} = \frac{\text{steady state plasma concentration} \times \text{clearance}}{\text{pump delivery rate}}$$

In this equation, the steady state plasma concentration is the desired concentration that the pharmacologist intends to investigate and the clearance value is either estimated in silico or obtained from a previous pharmacokinetic study in the same species. It is important to note that while this equation is more frequently used to estimate steady state concentrations following administration by intravenous infusion, it can still serve as a simple method for approximating concentrations derived from continuous subcutaneous infusion as well. In doing so, an assumption must be made that the bioavailability following subcutaneous administration is 100 % relative to an intravenous dose.

Unlike subcutaneous bolus injections that can be formulated as suspensions in isotonic vehicles, only solution-based formulations are acceptable for osmotic pump delivery. However, given the small volumes delivered through the pump, high concentrations of nonaqueous solvents may be used, as long as they are water miscible and used in amounts that are compatible with the pump components. Alzet infusion pumps are known to be compatible with a wide variety of different types of media, and in the absence of available solubility data for the test compound in these media, extensive screening may be required to identify the optimal formulation. In practice, however, the screening and selection of a formulation may be done more efficiently and with less compound by evaluating an abbreviated list of solvent systems, generally categorized by the amount of organics present, and thus the

overall aggressiveness of the formulation. For example, a typical set of solvent systems would likely include at least one aqueous-based system as well as a 1:1 mixture of an organic (i.e., DMSO) with water, and a very aggressive formulation consisting entirely of organics. Based on the results of this initial screening, further formulation optimization may be applied as needed. This approach allows for conservation of material which is often very limited at this stage of development. The formulation thus developed is then tested for precipitation potential during delivery. This is of critical importance since precipitation can result in clogging of the pump resulting in a complete failure of the study. This may be accomplished by simply filling the pumps with the proposed formulation(s) and incubating them in normal saline or a blood surrogate buffer and then monitoring the appearance of compound in the media as a function of time. Additional studies may also be performed to test the chemical stability of the compound in the selected formulation and compatibility with pump components if needed (Gullapalli et al. 2012).

2.4.2 In-feed Dosing

Sustained plasma exposures for pharmacology studies can also be achieved using in-feed dosing options. This approach is based on the fact that rodents eat at frequent intervals and their feeding patterns through the light and dark phases of the day are well understood. This information, along with the desired plasma concentration and clearance data, makes it possible to calculate the amounts of compounds to be incorporated into their feed. Both solid and liquid diets may be used, and combinations of multiple compounds may be dosed simultaneously with the feed as desired. Formulation of the active compound(s) with the feeds may be done in-house, or through labs that offer these services (Research Diets). As rodents eat approximately the equivalent of one tenth their body weight of food every day, the fraction of active drug in the feed is fairly small (e.g., about 0.2 % for a dose of 200 mg/kg).

In-feed dosing offers several advantages over continuous infusion pumps. It eliminates the need for solubilization of compounds in small volumes of formulation solvent and the associated risk of precipitation. It minimizes handling of the animals for implanting the pumps (and the wound healing process that follows) and allows for significantly longer term dosing. Lastly, with compounds that cause injection site irritation, or for pain and inflammation projects that want to avoid the injury caused by pump implantation, this is the preferred option for achieving sustained plasma concentrations of test compounds. One example of the application of in-feed dosing is with sitagliptin, a DPP-4 inhibitor that has a short half-life of 1–2 h in mice. Mu et al. (2009) were able to demonstrate chronic glycemic control over 10 weeks, with in-feed dosing of 280 mg/kg of this compound in mice (equivalent to 0.3 % w/w of the mouse diet). Important considerations in using this type of formulation include variability in exposure due to eating, binding to food, assurance of homogeneity of dosage form, and stability.

2.5 Pharmacokinetic Studies

Greater emphasis is increasingly being placed on early in vivo characterization and evaluation of key compound pharmacokinetic properties in order to select molecules that possess that greatest likelihood of long-term clinical success. After initial screening through batteries of in vitro biochemical and physicochemical assays, promising compounds are typically evaluated in a single-dose pharmacokinetic (PK) study, usually in a rodent species such as the rat. These studies are designed to include both an intravenous arm, as well as a second arm that approximates the intended route of administration in man, usually oral. The primary goal of these studies is to filter compounds with poor ADME characteristics, as well as to begin to develop a more mechanistic understanding of these properties in order to influence the SAR toward design of better molecules.

One of the more challenging aspects of conducting a pharmacokinetic study is the identification of a suitable vehicle to be used to solubilize the compound for use in the intravenous arm of the study. In the early discovery setting, compounds selected for testing often possess suboptimal physical properties (i.e., low solubility, high log *P*). In addition, the relatively large number of compounds selected for in vivo testing, as well as the need for very rapid data turnaround, presents significant challenges in screening and evaluating potential IV vehicles. A number of general approaches for the identification of a suitable IV formulation have been published previously. These approaches typically involve a very methodical screening of a variety of different options until a suitable solution is found. Lee and coworkers (2003) proposed a decision tree for use in early discovery that allows for selection of a suitable formulation using observations of experiments in which various pH and cosolvent concentrations are tested, based on the underlying physicochemical properties of the molecules in question. Similar approaches have been utilized across the pharmaceutical industry. In practice, however, these types of approaches are often impractical in the discovery setting, due to limitations on material for analysis and testing, large numbers of compounds under consideration, and challenging time constraints. In keeping with the formulation strategy described previously, the formulation scientist must be able to make formulation decisions by eliminating as many options as possible based solely on the properties of the molecules in question (i.e., p*K*a, log *P*, MW, melting point) and then rely on very limited in vitro screening to narrow the list of potential vehicle options. Initial attempts at solubilization often rely on the use of a complexing agent, such as a cyclodextrin in either an acidic or basic aqueous buffer solution, depending on the ionization properties of the molecule in question. Compounds that are not amenable to solubilization in these vehicles are then evaluated in more aggressive vehicles containing increasing amounts and types of organic cosolvents. It is important to note that these more aggressive vehicles, while well tolerated in single-dose studies, are not likely to be compatible with repeat dosing due to toxicity following chronic administration of the excipients. While solubilization is the primary goal of these

studies, it is also important to evaluate the potential for precipitation of the compounds from the solubilizing vehicle. Several publications have described the use of in vitro precipitation screens to study precipitation from injectable formulations (Sheth 2011). Several in vitro screening methods are described, one utilizing static dilution into aqueous media and the other evaluating dynamic injection. These screens were used to assess the potential of in vivo precipitation of nine injectable formulations, which were selected from marketed products as well as several from internal Merck research programs. Good correlation was observed between results from the static and dynamic models. In addition, the in vitro data was found to correlate with instances of precipitation that were noted during preclinical testing in animals as well as clinical testing.

In addition to developing injectable formulations for individual compounds, the need to rapidly evaluate pharmacokinetic properties for larger numbers of compounds has led to the use of different approaches which have been proposed to improve the throughput and efficiency of these studies. One of the most promising techniques is cassette dosing, or N-in-1-dosing, which involves simultaneously administering a set of compounds in a common vehicle, as opposed to discrete dosing in which a single compound is administered (Nagilla et al. 2011). This approach allows for a reduction in the number of both studies and animals required, as well as the generation of fewer samples for bioanalytical analysis. Several independent analyses have demonstrated that PK parameters obtained through cassette dosing are comparable to those derived from dosing compounds (He et al. 2008; Nagilla et al. 2011) discretely. Despite these advantages, careful consideration must be given to the experimental design when choosing to conduct in vivo studies using cassette dosing. The identification and selection of a common vehicle for a series of structurally unique compounds can be challenging. When possible, compounds should be grouped together by structural class in order to take advantage of common chemical features. Compounds within a given class may behave similarly in terms of the mechanism and extent of solubilization. Combining compounds with significantly different functionality (i.e., mixing acidic and basic compounds) should generally be avoided, as these differences will likely make the identification of a suitable common vehicle very difficult if not impossible.

In addition to intravenous delivery, the administration of an oral dosage form is also included as a second arm in a typical pharmacokinetic study. Some minimal threshold for oral bioavailability (i.e., 20–30 %) is then used as filtering criteria in an effort to identify and de-prioritize compounds or structural classes that possess undesired absorption risks. This approach to selecting compounds to advance is problematic for a number of reasons. A recent report comparing measured animal (mouse, rat, dog, primate) and human bioavailabilities of 184 compounds extracted from the literature showed a very poor correlation (Musther et al. 2013). Additionally, the solid state properties of molecules are not typically controlled or even fully characterized in the discovery phase. As a result, the solubility and resulting

bioavailability often decrease when subsequent lots of material become available which are more crystalline. Moreover, the doses selected for pharmacokinetic studies are often not reflective of what will be used in first in human trials. At lower doses, the absorption of compounds is often rapid and complete, even for compounds with low solubility. However, at higher doses, solubility and/or permeability limitations will begin to negatively impact absorption, resulting in oral bioavailabilities significantly less than the original target values. In general, to properly use this approach, several important aspects should be considered beyond simply using this threshold as a means to filter compounds. Developing a deeper understanding of the root cause of low bioavailability provides important feedback to the discovery team so that additional hypotheses can be proposed and tested in an attempt to resolve these issues. From the standpoint of the formulation scientist, it is critical to understand what impact formulation may have on bioavailability in order to correctly identify absorption risks and apply enabling formulation strategies when appropriate. Experimental approaches to diagnosing the cause of limited absorption represent the topic of Chap. 4, but are also treated below.

Bioavailability (%F) is defined as the product of absorption and metabolism and is represented by the following equation:

$$F = \mathrm{Fa} \times \mathrm{Fg} \times \mathrm{Fh}$$

where Fa = fraction absorbed, Fg = fraction escaping gut metabolism, and Fh = fraction escaping hepatic metabolism. Consider two hypothetical compounds, A and B (Fig. 2.7). Upon oral administration, a significant amount of Compound A remains unabsorbed, while Compound B is almost completely absorbed. However, first-pass extraction by either gut and/or liver metabolism is relatively minimal for Compound A while Compound B is significantly metabolized. As a result, both Compounds A and B would be found to have similar relative oral bioavailabilities. However, it is clear from this simple example that the underlying absorption and metabolism properties of the two molecules are quite different. Further analysis and additional experimental data may be needed to fully elucidate these differences. For Compound A, improvements in solubility and/or permeability or the use of enabled formulations should be targeted as a means to increase Fa, while for Compound B, additional SAR effort would be required to reduce metabolism of subsequent compounds. In cases where bioavailability is determined to be limited by Fa, it is necessary to distinguish between solubility and permeability limited absorption. In the discovery phase, this is often relatively straightforward due to the availability of in vitro systems designed for this purpose (Caco-2, MDCK, etc.). Once solubility has been identified as the primary issue, there is a need to further interrogate whether this is related to poor dissolution or low solubility. For compounds that are dissolution rate limited, micronization, either by milling the neat API or by probe sonication of a suspension, will result in enhanced bioavailability. The impact of particle size reduction on absorption may also be assessed using

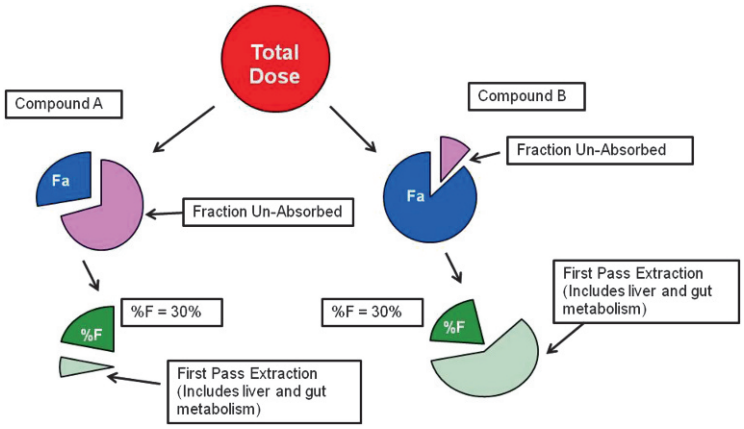

Fig. 2.7 Comparison of oral bioavailabilities of two hypothetical compounds A and B

computational approaches, such as the microscopic mass balance model described by Oh et al. (1993). In addition, commercially available software such as GastroPlus from Simulations Plus, LLC may be used to simulate the effects of changing particle size on absorption. Further reduction in particle size may be achieved through the production of nanosuspension formulations. If micronization alone is found to be ineffective in improving the dissolution properties of a compound, the addition of a surfactant such as polysorbate 80 may be added to improve the wetting properties of the material. In practice, particle size reduction is typically combined with the use of low levels of a surfactant in the formulation at the outset. When this approach still leads to lower than desired exposures, the use of solution-based formulations is then employed. This approach can range from the very simple, such as pH adjustment, to the use of complexing agents and cosolvents, and even to the development of stabilized amorphous formulation such as solid dispersions. When using these types of formulations for pharmacokinetic studies however, it is important to keep in mind the original goals of the study and to carefully evaluate the impact of the dosage form on the interpretation of the resulting data. A brief listing of general considerations for solutions and suspensions is presented in Table 2.5. For example, in cases where the type and/or extent of metabolism is being investigated, it may be desirable to utilize a solution formulation as a way to eliminate any impact of the solid state properties of the molecule. However, when the aim is to develop an understanding of the absorption properties of a solid oral dosage form, and thus gain insight into possible future development challenges, dosing of a suspension is preferred so as not to mask any absorption risks due to poor physicochemical properties. The exposure obtained following administration of a suspension in a pharmacokinetic study also provides an early indication of the likelihood of achieving sufficient exposures in subsequent toxicology studies, in which case the development of an enabled formulation might

Table 2.5 General properties of solution and suspensions

Solution	Suspension
Drug is completely dissolved	Drug is suspended homogeneously as fine particles
Drug directly available for absorption	Dissolution is necessary before drug becomes available for absorption
Unless properly formulated, drug might precipitate upon dosing	Rate of dissolution is a function of particle size and solubility
Solubility is a limiting factor for formulation; dose volume may be limiting factor due to excipient toxicity	Solubility is not a factor for formulation, but formulation viscosity may limit ability to use higher doses
Required for intravenous dosing; may be dosed orally to eliminate impact of solid state	Required when goal of study is to better understand oral bioavailability and absorption properties; solid state properties must be carefully considered

be required. It is important to note that if the data is to be used to draw conclusions about absorption, the solid state properties of the material used in the animal studies should be as representative as possible of the form that would be progressed in development. If the data is generated early in the project's lifecycle using material where solid state properties are either unknown or are found to be dramatically different than subsequent lots of material, exposure studies should be repeated with more representative material to ensure that the impact on absorption is characterized. Given this caveat, a reasonable argument can be made that in early discovery, it is not always appropriate to include an oral arm in a basic pharmacokinetic study, as the resulting data may not be relevant and may even at times result in absorption risks being over or underestimated.

Another key factor that must be considered in selecting a formulation is the potential for the formulation excipients to alter the pharmacokinetics of the test compound. The presence of suboptimal physical properties necessitates the use of vehicles containing organic cosolvents, cyclodextrins, and surfactants. A thorough review of the effects of common excipients on ADME properties was published by Buggins et al. (2007). Table 2.6 provides a summary of doses at which minimal effects are to be expected for a subset of the most commonly used excipients. For each route of administration, a maximum dose volume is specified at which the excipient's effect on the pharmacokinetics is expected to be minimal based on a relatively exhaustive search of the literature.

2.5.1 Absorption Modeling of Animal Pharmacokinetic Data

The underlying assumption in using preclinical species to conduct pharmacokinetic studies is that the results of these studies will have some relevance to absorption in

Table 2.6 Dose volumes of common pharmaceutical excipients at which minimal effects on pharmacokinetic properties are expected

Excipient	Recommended levels for in vivo studies
DMSO	IV and PO: Max 5 % DMSO with dose volume of 5 mL/kg (0.2 mL/kg DMSO)
EtOH	PO: Max 10 % with dose volume 10 mL/kg (1 mL/kg EtOH). Chronic dosing can influence PK due to effect on enzymes.
Propylene glycol	IV and PO: less than 3 mL/kg for pharmacology studies measuring plasma glucose levels.
PEG400	PO: Max 40 % PEG400 in formulation with 5 mL/kg dose volume (2 g/kg rats).
	IV: 40 % PEG400 at 1 mL/kg (0.4 mL/kg PEG400). Known inhibitor of drug efflux and also CYP3A, thus may enhance absorption of such compounds that are substrates.
HPβ cyclodextrin	PO: Max 20 % if 10 mL/kg (2 g/kg HPBCD).
	IV: Max 20 % if 2 mL/kg, 400 mg/kg HPBCD.
	Effect on distribution depends on protein binding, stability constant of complex.
SBEβ cyclodextrin	IV: Max 20 % if 4 mL/kg
Cremophor EL	IV and PO: Increased absorption due to inhibition of P-gp and CYP3A4, inhibits absorption by micellar entrapment.
PS 80	PO: Max 0.5 % if 10 mL/kg (0.05 g/kg PS 80).
Solutol HS 15	IV: Max 5 % with dose volume of 2 mL/kg.
	If compound is a P-gp substrate, may significantly alter PK after either IV or PO administration.
	Data suggest that Solutol or a component there-in is absorbed orally, PK of IV administered drug altered after PO administered Solutol HS 15.

humans. Chiou and Barve (1998) conducted studies to investigate the extrapolation of absorption experiments in rodents to humans. In this study, 64 compounds were selected from the literature where data existed for both species. Despite the fact that the compounds possessed a very broad range of physicochemical properties, including molecular weight (150–4,000), ionization state (acids, bases, neutral), and lipophilicity ($\log P$ −5 to +4), an excellent correlation was observed between absorption (Fa) in rats relative to humans ($r^2 = 0.975$). However, a very poor correlation was observed when a similar analysis was conducted to compare estimation of human bioavailability based on rat studies. This was attributed primarily to differences in expression of metabolizing enzymes in the intestines of the two species (Fg and Fh). Physiological differences between species must also be carefully considered both in the experimental design as well as in the interpretation of the data. For example, gastric pH has been shown to vary considerably in the dog, with values of fasting gastric pH reported to range from 1.8 to as high as 6.8 (Lui et al. 1986; Yamada and Haga 1990; Akimoto et al. 2000). In humans, however, gastric pH has consistently been shown to be less than 3, regardless of

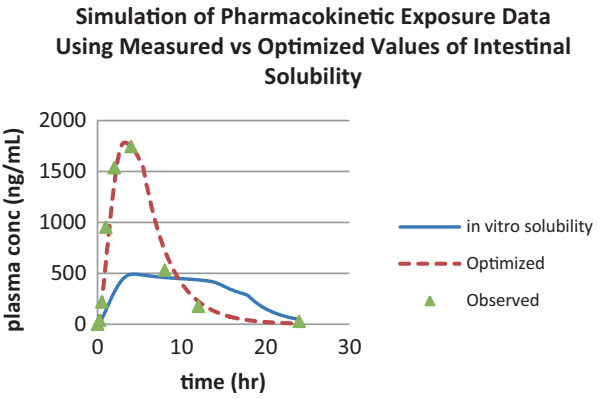

Fig. 2.8 Comparison of GastroPlus™ simulations using either in vitro solubility or optimized solubility to represent intestinal solubility of the test compound

the method used to measure it (Fancher et al. 2011). As a result of these differences, caution must be used in using the dog as a model of human absorption, especially for test compounds with ionization constants in the range of 5–8. To overcome this issue, the use of pentagastrin is common to control the canine stomach in a range that is more relevant to human fasting conditions, while the proton pump inhibitor (PPI) famotidine is recommended to simulate elevated stomach pH conditions.

Estimation of absorption risk in the discovery setting is a key activity that provides discovery scientists with a relatively straightforward method of selecting scaffolds that will ultimately achieve sufficient oral exposure to allow for testing of the clinical hypothesis. Once exposure data becomes available from rodent and/or canine pharmacokinetic studies, absorption modeling using commercial modeling packages such as GastroPlus™ may be used to form an initial assessment of absorption potential and risk. This approach requires a minimal set of measured parameters, which can be obtained from both in silico tools as well as experimentally. These parameters serve as inputs to the software, which when combined with prebuilt physiological variables, allow the user to evaluate an initial fit of the experimental plasma concentration data. In the event a good fit is not obtained, key parameters related to absorption (i.e., solubility, permeability, particle size, etc.) may then be optimized until an adequate fit is obtained. An example is highlighted in Fig. 2.8. Fitting of the observed exposure data was not possible using a value for intestinal solubility taken directly from an in vitro solubility experiment in fasted simulated intestinal fluid (FaSSIF). Optimization of the solubility parameter resulted in the estimation of an in vivo solubility that was significantly higher than the in vitro value. These optimized values may then be used to estimate absorption potential in humans, simply by applying a human physiology to the existing model (Fig. 2.9). This general method allows for rapid assessment of compounds and provides a means of either eliminating compounds with poor absorption potential or mitigating exposure limitations through the use of enabled formulations.

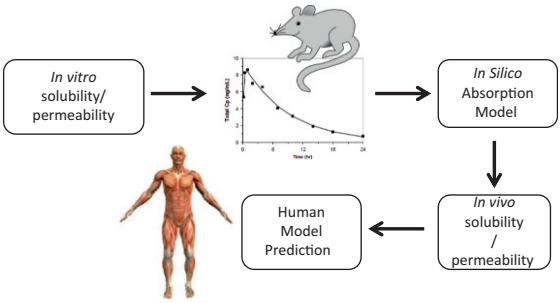

Fig. 2.9 Absorption modeling of in vivo pharmacokinetic data as a method for estimating human absorption risk

2.6 Toxicology Formulations

Toxicology or nonclinical safety assessment is a critical component of the discovery and clinical development of any pharmaceutical agent. There are many excellent reviews regarding toxicology testing in drug discovery and development and the reader is encouraged to consult these papers to gain a thorough understanding of toxicology studies necessary to support human dosing (Dorato and Buckley 2007; Buckley and Dorato 2009; Higgins et al. 2012). Regulatory guidance for conducting nonclinical safety studies have also been issued (M3(R2)). The safety term often used in the support of clinical trials is the Margin of Safety (MOS). The MOS relates to the No Observed Adverse Effect level (NOAEL—a dose that produces no relevant adverse effects) to the maximum effective dose and is displayed in Fig. 2.10. Dosing up to the maximum tolerated dose (MTD) to demonstrate target organ or dose limiting toxicity is a general expectation of regulatory authorities in support of clinical testing. In the absence of this, other equally appropriate dose-limiting criteria may be considered. These include a limit dose (1,000 or 2,000 mg/kg) that results in acceptable exposure margin relative to the clinical dose, a 50 fold exposure multiple, or an exposure limiting dose or maximum feasible dose (ICH M3(R2)).

The toxicology formulation must provide consistent plasma exposures with low variability and clear dose separation. This must be accomplished using excipients that have adequate safety data supporting the amounts used and the duration of the study. Conventional suspension formulations with particle size control, prepared in standard vehicles that are well understood and characterized in terms of safety, are preferred for oral toxicology studies and will ideally provide a linear increase in exposure with dose as depicted in Fig. 2.10. However, it is not uncommon for a compound to display solubility limited absorption in the toxicological dose range from a standard suspension vehicle, resulting in a plateau in the dose versus exposure relationship. If no dose limiting toxicity is observed, then avenues to enhance systemic exposure and/or justify a maximum feasible dose must be explored.

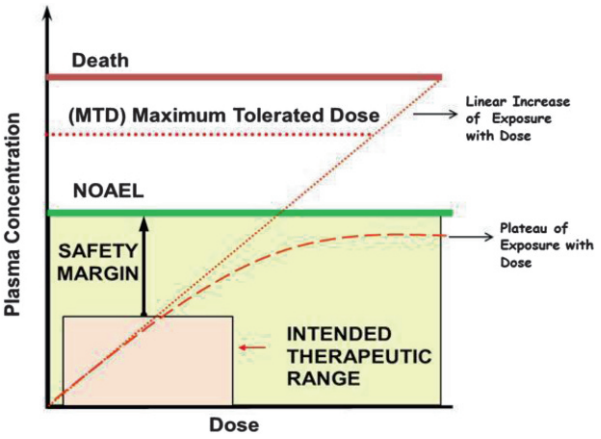

Fig. 2.10 Plasma concentration verse dose relationship in toxicology testing. Ideally, the formulation provides a linear increase in exposure with dose. Solubility limited molecules may exhibit a plateau of exposure with dose

Early alerts to the potential for exposure related issues at toxicological doses and a strategy for how and when to apply various formulation approaches are critical to avoid delays in the program timelines. Basic molecular properties that may be indicative of solubility limited absorption are listed in Table 2.7. The pharmacokinetic and absorption models previously described are particularly useful tools to understand absorption sensitivity (e.g., particle size, solubility, pH) and to identify early risk of saturation of exposure as dose is increased. It is not until actual studies are conducted however that dose dependency can be more fully understood. For example, in repeat dose-exposure response studies (e.g., toxicokinetics from a 4- or 14-day study), enzyme auto-induction may be observed as a confounding factor in hepatic clearance and may be mistaken for an absorption-limited phenomenon.

Once a plateau is observed in either predicted or observed exposure (in absence of a dose limiting toxicity), an appropriate amount of due diligence is needed to improve exposure. A strategy for toxicology studies is illustrated in the pyramid (Fig. 2.11). Two categories of options can be utilized; study design options involve the modification of dosing parameters (e.g., frequency and route of administration). Formulation options involve application of Alternative formulations that address the root cause of absorption issues. Considerations influencing the decision on which option to use include ease of implementation, cost, and/or logistics. A detailed review of the advantages and disadvantages of various options is provided in Table 2.8. Most pharmaceutical companies explore a number of options (formulation, dose frequency) to achieve higher exposure. The rationale and supporting data for the recommended strategy should be well documented to support regulatory submissions if needed.

The overall complexity of each method is represented by its relative height on the pyramid in Fig. 2.11. This ordering of formulation options is not meant to imply that each method must be tested in succession, starting from the bottom and working toward the top. Rather, key information as to the type and goal of the study, as well as physicochemical properties (e.g., lipid solubility, ionization) should be taken into account in order to determine the most appropriate level in

Table 2.7 Parameters suggesting solubility-limited absorption

Properties	Alerts
p*K*a	No ionizable groups
	Weak base (p*K*a < 4)
	Weak acid (p*K*a > 5)
Log *P*	>5
Melting point	>250 °C
Solubility in fasted simulated intestinal fluid (FaSSIF)	≤100 μg/mL (dependent on permeability and dose (Willmann et al. 2004)
Fraction absorbed (calculated) (rodent and nonrodent)	<0.2 (dosed as a solution or suspension at pharmacology dose, permeability limited absorption ruled out)

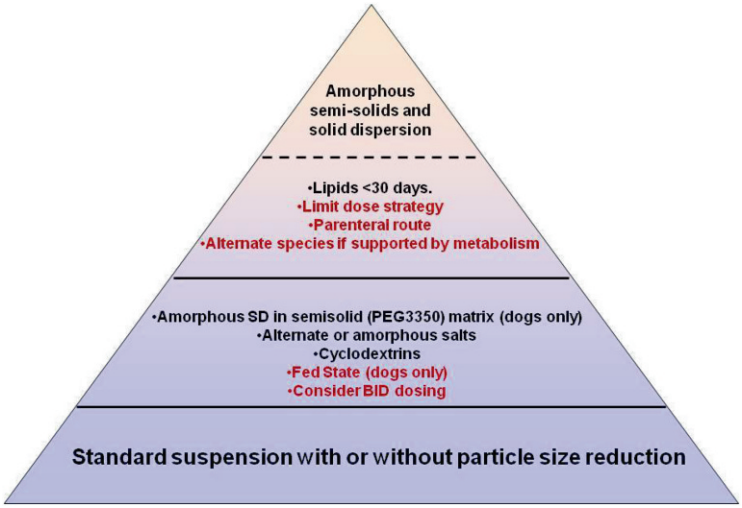

Fig. 2.11 The "pyramid" of dosing options or study design to increase exposure in toxicology studies

which to start. If one option fails, moving to the next level of complexity may be warranted in order to identify a suitable solution. This combined with in vitro dissolution tests such as the artificial stomach and duodenum and computational modeling can serve as valuable tools to choose the best formulation approach. The dissolution tools can be used to assess the rate and extent of dissolution, and also precipitation/supersaturation, properties of formulations under biorelevant conditions. Computational modeling tools can be used to identify the relative impact of absorption parameters (e.g., solubility, particle size, permeability, etc.) on exposure limitations (Bhattachar et al. 2011).

Solid dispersions are at the peak of the pyramid shown in Fig. 2.11. The use of solid dispersions has been very successful at Lilly for toxicology testing and this formulation option has provided high exposures for solubility limited molecules. An added advantage of solid dispersions is the clear "line of sight" to the clinical

Table 2.8 Summary of options to enhance toxicology exposure

Options	Advantages	Disadvantages
Study design		
b.i.d. dosing regimen	• Enhanced exposure Css (if appropriate $t_{½}$)	• Greater potential for stress-induced pathology and dosing accidents
		• Complicates kinetics and evaluation of exposure
	• Conventional formulation	• Increased compound requirements
		• May confound direct correlation to human QD dosing
Dose in fed state vs. fasted state (dog only)	• Conventional formulation	• Difficulty to control food intake
		• Pharmacological effect might affect food consumption during the study
		• Potential for variability in exposure
Limit dose strategy (e.g. 1,500 mg/kg)	• Conventional formulation	• High compound requirements
		• Potential safety issue if lack of premonitory signs for toxicity complicates clinical plan. Dose to exposure not MTD; requires real-time analysis of plasma and PK, and slower escalation
		• Acceptable MOS achieved, no MTD
		• Limit dose may not be deemed adequate by regulatory authority (e.g., certain divisions at FDA)
Increase dose volume[a]	• Conventional formulation	• Marginal improvement of AUC. May increase variability due to effects on GI motility or emesis
Change route of administration to define toxicity (e.g. IV infusion)	• Potentially higher systemic exposure with lower doses	• Potentially altered metabolite profile which may alter MTD or observed effects
		• Enabling vehicles still required for low-solubility compounds
		• Complicates long-term toxicology study designs as multiple routes may need to be tested
		• Route differs from clinical route
Change rodent or nonrodent species	• Enhance exposure	• Potentially altered metabolic profile compared to humans
Formulation		
Different salt form	• Enhance exposure	• Bridging studies between salt forms may be necessary

(continued)

Table 2.8 (continued)

Options	Advantages	Disadvantages
Toxicology vs. first in human studies		• Depending on the impurity profile of salt, may extend FHD timelines. New salts (other than those approved by FDA) may require additional long-term data
		• If amorphous, potential to crystallize in suspension
Solubilizing vehicles (lipid/cosolvent/ surfactant)	• Can be prepared in toxicology and contract labs.	• Lack of experience with prolonged dosing
		• Limited dose volume and dose
		• Effect on pharmacokinetic parameters
		• Stability in presence of oxidizing excipients
		• Emesis, particularly in dogs
		• In vivo precipitation
		• May require a placebo arm
Solid dispersions	• Generally involves GRAS excipients	• API stability required to support long-term toxicology studies
	• High potential of significantly increasing exposure when designed appropriately for highly crystalline compounds	• Maximum loading dose limited (approximately 25 %)
		• Residual solvents
		• Requires sufficient characterization to assure chemical and physical stability of the solid form
		• Physical stability in suspension sufficient for dosing
Nanoparticles	• Use of conventional excipients	• May not enhance exposure if solubility limited.
		• Concentration of surfactant to stabilize nanosuspensions should be acceptable for long-term toxicology studies
		• Particulates from bead or equipment
		• Increase in particle size due to Ostwald ripening, hold time limits may be required

[a]Increasing dose volume may lead to physiological changes such as reflux, fasting, and alterations in gastrointestinal transit. Dose volumes higher than the recommended amount may be considered under the guidance of veterinary resources

dosage form since it can be used for both animal and human testing. A drawback is the additional resources and API is required for polymer screening, analytical testing (physical and chemical), and the use of larger scale specialized equipment

(e.g., spray drying). However, the current state in understanding amorphous systems has improved in recent years and the development of automation and small-scale laboratory equipment to perform polymer screening/solid dispersion manufacture is making this an easier dosage form to execute (Friesen et al. 2008; Nagapudi and Jona 2008). Small-scale manufacture of amorphous dispersions is thoroughly discussed in Chap. 3.

As evident from Fig. 2.11 and Table 2.8, the use of nanoparticles, SMEDDS, and cosolvents in toxicology studies may be somewhat limited due to the following reasons.

Nanoparticles—The use of nanoparticles to enhance bioavailability at moderate doses is well recognized and there are commercial products that utilize this technology. At toxicological doses where absorption is solubility limited, these approaches are less successful based on Lilly experience. There are limited examples of the use of nanoparticles at high toxicological doses (Kesisoglou and Mitra 2012). The use of computational modeling tools to predict the exposure increase that can be achieved with this approach is somewhat limited at this time, as the in vivo absorption parameters of nanosuspensions are not very well understood.

SMEDDS (Self Microemulsifying Drug Delivery Systems) and Cosolvents—The amounts of surfactants and cosolvents necessary to achieve the solubility enhancements typically required in toxicology studies are generally poorly tolerated due to local gastrointestinal effects, and consequent effects on electrolytes and body weight over time. Thus, their use here has been limited to short-term exploratory studies.

Cosolvents—Formulations based on cosolvents may be used in small amounts for shorter term studies that generally do not exceed 4 days (Lilly internal experience) and thus these excipients are not considered to be preferred options. However, they are fairly simple to prepare and their use must always be based on a good understanding of the risk of precipitation upon administration.

Several decision trees for toxicology formulation development to conserve resource have been proposed in the literature (Higgins et al. 2012). Acceptable toxicology vehicles have also been previously published (Brewster et al. 2007). Additionally, a key resource can be an in-house database that archives details on different toxicology studies. This database could contain information on the API characteristics, formulation approach, exposure enhancement attained, and adverse effects reported in the animal model. Additional resource is the Vitic Excipient Database—Lhasa LTD as mentioned earlier.

As toxicology studies use large amounts of compound, the formulation and design options used in these studies have significant implications on the amounts of compound required, and this in turn can impact cost and timelines. Some enabled formulations may need slightly longer time and larger amounts of material to be made available for formulation and process development work. Studies using enabled formulations may help reduce the administered doses by improving the fraction absorbed, but depending on the type of formulation, there might be processing or handling losses incurred that need appropriate planning. Therefore, regardless of the formulation or study design options used, effective collaboration

between toxicologists, formulators, and chemists is essential for planning and successful execution of toxicology studies.

2.7 Formulation Considerations for Alternate Drug Delivery

Alternate formulations or delivery routes are widely utilized as a means to modify the process of administration or in vivo release profile of pharmaceutical agents. Efforts to develop these formulations are often initiated late in the development process as part of a lifecycle management strategy (Chien and Ho 2011). In more recent times, however, successful drug delivery strategies have actively assessed alternate drug delivery systems in parallel with molecule selection, when molecule attributes may still be influenced, to help ensure the evolution of developable systems that meets the patients' and product lifecycle needs.

Alternate drug delivery may be particularly important for patient populations with elevated needs around cognition, behavior, or dexterity, including therapeutic areas such as Alzheimer's (Muramatsu et al. 2010), Parkinson's (Wright and Waters 2013) chronic and acute pain, and epilepsy (Anderson and Saneto 2012). Important therapeutic benefits and advantages may include a more favorable efficacy profile and/or alleviation of side effects. Examples include rivastigmine transdermal system, a cholinesterase inhibitor indicated for dementia of the Alzheimer's type and dementia associated with Parkinson's disease. The patch formulation provides an improved gastrointestinal side effect profile compared with oral administration (Exelonpatch.com) and caregiver convenience and preference for use (Bernabei, Rossini et al. 2012). Rotigotine, a dopamine agonist indicated for Parkinson's disease, is only available by transdermal patch form as a means to provide continuous delivery over 24 h. Continuous rather than pulsatile delivery is believed to more closely mimic physiological dopaminergic stimulation (Waters 2013). Intranasal sumatriptan and intranasal fentanyl provide more rapid onset for acute migraine and cancer breakthrough pain, respectively, with time to onset of 10–15 min (Dietrich and Gums 2012).

Alternate drug delivery may also play a role in the delivery of pharmaceutical doses that could not be administered effectively or safely through conventional routes. Sublingual tablets and sprays for nitroglycerin avoid extensive first-pass metabolism and provide rapid onset for treatment of angina pectoris. Similarly, nonoral administration of testosterone avoids the high first-pass metabolism and hepatotoxicity following conventional oral delivery and allows therapeutic exposures to be achieved (Pfeil and Dobs 2008). Marketed testosterone delivery systems include topical, transdermal patch, buccal, and IM depot (prodrug).

The decision to purse alternate drug delivery can occur at any stage of the drug discovery, development, or commercialization lifecycle. Early awareness by teams

Table 2.9 Preferred physicochemical properties for various routes of delivery.

Criteria	Intranasal	Pulmonary	Transdermal	Sublingual/buccal
MW	<1,000	<10,000	<500 (<350 preferred)	<500
Log *P*	1–4	−1 to 2	2–4	2–4
p*K*a	4–9	4–9	Unionized	4–9
pH range	4–7	3–7	4–7	3–8
Volume	50–150 μL	<200 μL	5–10 μL/cm^2	<500 μL

of potential benefits of alternate drug delivery and guidance around parameters required for various routes of administration can help to shape robust development strategies. This generally begins with cross-functional team discussion of the target product profile and potential therapeutic opportunities. Key questions center on:

1. Therapeutic benefits for the patient.
2. Efficacy and adverse effect profile and relationship to C_{max} and/or AUC.
3. EC_{50} or minimum concentration required to exert a therapeutic effect.

Once potential opportunities are identified, an initial assessment of feasibility can often be made with minor adjustments to the computational and formulation screens described previously. The physicochemical properties generally preferred for common routes of delivery have been reviewed (Mathias and Hussain 2010) and key parameters are summarized in Table 2.9. Further details may be found in recent reviews for transdermal (Neely et al. 2009; Paudel et al. 2010; Watkinson 2013), intranasal (Chapman et al. 2013), and sublingual/buccal delivery (Zhang et al. 2002; Goswami et al. 2013; Lam et al. 2013). Strict limitations around human efficacy dose (Table 2.10) for nonoral routes of administration are due primarily to permeation limitations or dose volume constraints at these sites. Consequently, good estimates of human efficacy dose and understanding of pharmacokinetic parameters as described for ADME studies are critical.

Equally important is ready access to formulations that allow exploration of pharmacokinetic and pharmacodynamic response in in vivo studies. Compared with oral administration, the range of vehicle options is more limited due in part to the high exposure to the excipients at the application site. Table 2.11 lists examples of vehicle systems employed for screening studies by various routes of administration. These relatively simple formulations may serve as a baseline for future formulation optimization studies. When possible, it is helpful to select excipients and concentrations with a history of prior use in humans so as to not over-enable exposures or generate adverse local effects. Good resources for this information include the FDA Inactive Ingredient Guide (FDA Inactive Ingredient Guide), major compendia (e.g., USP/NF, JP, PhEur), excipient suppliers, and external databases such as Lhasa (Lhasa Vitic Nexus database).

Alternate drug delivery may add significant value to a candidate if therapeutic advantages are realized for the patient. Early assessments of potential opportunities and technical feasibility in the discovery phase may serve to provide realistic expectations prior to investment in more costly development activities.

Table 2.10 Maximum human doses and potential therapeutic benefits of different dosage forms and routes of delivery

Dose form with assumptions around total unit size or wt	Preferred dose (mg/dose)	Rapid onset	Sustained plasma exposure	Decrease dose frequency	Minimize first-pass metabolism
Oral					
Conventional tablet, 150–450 mg	<100	–	–	–	–
Sustained release (matrix or multiparticulate)	<50	–	√	√	–
Orodispersible tablet	<25	√	–	–	–
Orodispersible film strip, 150 mg	<25	√	–	–	–
Soft gelatin capsule, #0, 0.68 mL	<100	√	–	–	–
Fine granules (5 g sprinkles, 20 % active)	<1,000	–	–	–	–
Sublingual					
Tablet	<10	√	–	–	√
Spray (0.5 mL)	<10	√	–	–	√
Transdermal					
Passive patch (delivered dose)	<10	–	√	√	√
Gel (delivered dose, 5 g applied product)	<10	–	√	√	√
Injectable					
IM depot	<2 mL	–	√	√	√
Subcutaneous	<1 mL	–	√	–	√

Table 2.11 Standard vehicles for in vivo evaluation of alternate routes of delivery.

Route	Species	Volume	Formulation examples	References
Transdermal	Rat, monkey, minipig, and ex vivo studies	Typically 5–25 μL/cm^2 for pharmacology studies, up to 10 % of BSA.	(a) Hydroalcoholic gel: EtOH or IPA 60–85 %, water 15–40 %, propylene glycol 0–20 %, hydroxypropylcellulose 1–2 % to increase viscosity	Lee et al. (2010), Lehman and Raney (2012)
			(b) Ex vivo: PEG400 45 %/PBS 55 % pH 6.4	
Intranasal	Rat, dog	Rat 10–20 μL, Dog 100–150 μL	Aqueous buffers pH 4–9 or saline	Sutton et al. (1993), Blagg et al. (2007)
Sublingual/buccal	Dog	Dog up to ~1 mL	(a) Aqueous buffers pH 4–9	Gayrard et al. (2013)
			(b) Ethanol 40 % in water	

2.8 Summary

A basic tenet of any in vivo study depends on reliable delivery of the drug to the target site of action and is profoundly influenced by the formulation. Formulations can impact drug release, absorption, metabolism, and the PK profile. The pharmaceutical scientist at the discovery–development interface is best qualified and ideally positioned to recognize the unique formulation needs of discovery teams and provide the necessary support. The need for this support has steadily increased in recent years due to the growing sophistication of the discovery engine and the shift toward a chemistry space characterized by lower solubility, greater lipophilicity, and thus greater challenges with in vivo release and absorption. Delivery of these drugs by "traditional" means where the compound is dosed as a simple formulation in an aqueous medium is increasingly not an option for eliciting the desired pharmacodynamic response or toxicological exposure. Active engagement of the pharmaceutical scientist and the utilization of appropriate formulations for in vivo studies, while maintaining a clear line of sight to commercial development are essential to the success of any discovery program.

References

Akimoto M, Nagahata N et al (2000) Gastric pH profiles of beagle dogs and their use as an alternative to human testing. Eur J Pharm Biopharm 49(2):99–102

Anderson GD, Saneto RP (2012) Current oral and non-oral routes of antiepileptic drug delivery. Adv Drug Deliv Rev 64(10):911–918

Balazs O (2011) Preclinical formulation in early drug research. In: Tihanyi K, Vastag M (eds) Solubility, delivery and ADME problems of drugs and drug candidates. Budapest, Bentham, p 22

Bernabei R, Rossini PM et al (2012) Compliance and caregiver satisfaction in Alzheimer's disease: results from the AXEPT study. Dement Geriatr Cogn Dis Extra 2(1):418–432

Bhattachar SN, Perkins EJ et al (2011) Effect of gastric pH on the pharmacokinetics of a BCS class II compound in dogs: utilization of an artificial stomach and duodenum dissolution model and GastroPlus, simulations to predict absorption. J Pharm Sci 100(11):4756–4765

Bikiaris DN (2011) Solid dispersions, part I: recent evolutions and future opportunities in manufacturing methods for dissolution rate enhancement of poorly water-soluble drugs. Expert Opin Drug Deliv 8(11):1501–1519

Bittner BG, Bravo RC, Walter I, Huwyler J (2005) Impact of oral administration of the surface-active excipient Solutol HS 15 on the pharmacokinetics of intravenously administered colchicine. Lett Drug Des Discov 2(3):3

Blagg J, Allerton CM et al (2007) Design and synthesis of a functionally selective D3 agonist and its in vivo delivery via the intranasal route. Bioorg Med Chem Lett 17(24):6691–6696

Bravo González RC, Huwyler J, Boess F, Walter I, Bittner B (2004) In vitro investigation on the impact of the surface-active excipients Cremophor EL, Tween 80 and Solutol HS 15 on the metabolism of midazolam. Biopharm Drug Dispos 25(1):13

Brewster M, Claire M, Noppe M, Lampo A, Loftsson T (2007) The use of solubilizing excipients and approaches to generate toxicology vehicles for contemporary drug pipelines. In: Augustijns P, Brewster M (eds) Solvent systems and their selection in pharamceutics and biopharmaceutics. Springer, New York, pp 221–256

Buckley LA, Dorato MA (2009) High dose selection in general toxicity studies for drug development: a pharmaceutical industry perspective. Regul Toxicol Pharmacol 54(3):301–307

Buggins TR, Dickinson PA et al (2007) The effects of pharmaceutical excipients on drug disposition. Adv Drug Deliv Rev 59(15):1482–1503

Carino SR, Sperry DC et al (2006) Relative bioavailability estimation of carbamazepine crystal forms using an artificial stomach-duodenum model. J Pharm Sci 95(1):116–125

Carrier RL, Miller LA et al (2007) The utility of cyclodextrins for enhancing oral bioavailability. J Control Release 123(2):78–99

Chapman CD, Frey WH 2nd et al (2013) Intranasal treatment of central nervous system dysfunction in humans. Pharm Res 30(10):2475–2484

Charman SA, Perry CS et al (2006) Alteration of the intravenous pharmacokinetics of a synthetic ozonide antimalarial in the presence of a modified cyclodextrin. J Pharm Sci 95(2):256–267

Chaubal MV (2004) Application of drug delivery technologies in lead candidate selection and optimization. Drug Discov Today 9(14):603–609

Chen XQ, Gudmundsson OS et al (2012) Application of lipid-based formulations in drug discovery. J Med Chem 55(18):7945–7956

Chien JY, Ho RJ (2011) Drug delivery trends in clinical trials and translational medicine: evaluation of pharmacokinetic properties in special populations. J Pharm Sci 100(1):53–58

Chiou WL, Barve A (1998) Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. Pharm Res 15(11):1792–1795

Dafallah AA, al-Mustafa Z (1996) Investigation of the anti-inflammatory activity of Acacia nilotica and Hibiscus sabdariffa. Am J Chin Med 24(3–4):263–269

Dietrich E, Gums JG (2012) Intranasal fentanyl spray: a novel dosage form for the treatment of breakthrough cancer pain. Ann Pharmacother 46(10):1382–1391

Dimitrov NV, Meyer-Leece C et al (1996) Plasma alpha-tocopherol concentrations after supplementation with water- and fat-soluble vitamin E. Am J Clin Nutr 64(3):329–335

Dorato MA, Buckley LA (2007) Toxicology testing in drug discovery and development. Curr Protoc Toxicol Chapter 19: Unit 19 11

Dudley RE, Constantinides P (2011) Oral testosterone ester formulations and methods of treating testosterone deficiency comprising same. WIPO, Clarus Therapeutics, Inc

Fancher RM, Zhang H et al (2011) Development of a canine model to enable the preclinical assessment of pH-dependent absorption of test compounds. J Pharm Sci 100(7):2979–2988

Ferguson SA, Boctor SY (2009) Use of food wafers for multiple daily oral treatments in young rats. J Am Assoc Lab Anim Sci 48(3):292–295

Foster KA, Sun H et al (2013) Utility of gastric-retained alginate gels to modulate pharmacokinetic profiles in rats. J Pharm Sci 102(8):2440–2449

Friesen DT, Shanker R et al (2008) Hydroxypropyl methylcellulose acetate succinate-based spray-dried dispersions: an overview. Mol Pharm 5(6):1003–1019

Gad SC, Cassidy CD et al (2006) Nonclinical vehicle use in studies by multiple routes in multiple species. Int J Toxicol 25(6):499–521

Gao P, Guyton ME et al (2004) Enhanced oral bioavailability of a poorly water soluble drug PNU-91325 by supersaturatable formulations. Drug Dev Ind Pharm 30(2):221–229

Gao Y, Carr RA et al (2010) A pH-dilution method for estimation of biorelevant drug solubility along the gastrointestinal tract: application to physiologically based pharmacokinetic modeling. Mol Pharm 7(5):1516–1526

Gartner K, Buttner D et al (1980) Stress response of rats to handling and experimental procedures. Lab Anim 14(3):267–274

Gayrard V, Lacroix MZ et al (2013) High bioavailability of bisphenol A from sublingual exposure. Environ Health Perspect 121(8):951–956

Gleeson MP, Hersey A et al (2011) Probing the links between in vitro potency, ADMET and physicochemical parameters. Nat Rev Drug Discov 10(3):197–208
Goswami T, Kokate A et al (2013) In silico model of drug permeability across sublingual mucosa. Arch Oral Biol 58(5):545–551
Gullapalli R, Wong A et al (2012) Development of ALZET(R) osmotic pump compatible solvent compositions to solubilize poorly soluble compounds for preclinical studies. Drug Deliv 19 (5):239–246
Hageman MJ (2006) Solubility, solubilization and dissolution in drug delivery during lead optimization. In: Borchardt RT, Kerns EH, Hageman MJ, Thakker DR, Stevens JL (eds) Optimizing the drug-like properties of leads in drug discovery, vol VI, Biotechnology: pharmaceutical aspects. Springer, New York, pp 99–103
He K, Qian M et al (2008) N-in-1 dosing pharmacokinetics in drug discovery: experience, theoretical and practical considerations. J Pharm Sci 97(7):2568–2580
Higgins J, Cartwright ME et al (2012) Progressing preclinical drug candidates: strategies on preclinical safety studies and the quest for adequate exposure. Drug Discov Today 17 (15–16):828–836
Hopkins AL, Groom CR (2002) The druggable genome. Nat Rev Drug Discov 1:727–730
Jacquemin E, Hermeziu B et al (2009) Bioavailability of oral vitamin E formulations in adult volunteers and children with chronic cholestasis or cystic fibrosis. J Clin Pharm Ther 34 (5):515–522
Kesisoglou F, Mitra A (2012) Crystalline nanosuspensions as potential toxicology and clinical oral formulations for BCS II/IV compounds. AAPS J 14(4):677–687
Kesisoglou F, Panmai S et al (2007) Nanosizing—oral formulation development and biopharmaceutical evaluation. Adv Drug Deliv Rev 59(7):631–644
Kumar R, Knick VB et al (2007) Pharmacokinetic-pharmacodynamic correlation from mouse to human with pazopanib, a multikinase angiogenesis inhibitor with potent antitumor and antiangiogenic activity. Mol Cancer Ther 6(7):2012–2021
Kurkov SV, Loftsson T (2013) Cyclodextrins. Int J Pharm 453(1):167–180
Kurkov SV, Madden DE et al (2012) The effect of parenterally administered cyclodextrins on the pharmacokinetics of coadministered drugs. J Pharm Sci 101(12):4402–4408
Kwong E, Higgins J et al (2011) Strategies for bringing drug delivery tools into discovery. Int J Pharm 412(1–2):1–7
Lam JK, Xu Y et al (2013) Oral transmucosal drug delivery for pediatric use. Adv Drug Deliv Rev 73C:50–62
Larger PJ, Breda M et al (2005) Ion-suppression effects in liquid chromatography-tandem mass spectrometry due to a formulation agent, a case study in drug discovery bioanalysis. J Pharm Biomed Anal 39(1–2):206–216
Lee YC, Zocharski PD et al (2003) An intravenous formulation decision tree for discovery compound formulation development. Int J Pharm 253(1–2):111–119
Lee PH, Conradi R et al (2010) Development of an in silico model for human skin permeation based on a Franz cell skin permeability assay. Bioorg Med Chem Lett 20(1):69–73
Lehman PA, Raney SG (2012) In vitro percutaneous absorption of ketoprofen and testosterone: comparison of pluronic lecithin organogel vs. pentravan cream. Int J Pharm Compd 16 (3):248–252
Li P, Zhao L (2007) Developing early formulations: practice and perspective. Int J Pharm 341 (1–2):1–19
Li S, Pollock-Dove C et al (2012) Enhanced bioavailability of a poorly water-soluble weakly basic compound using a combination approach of solubilization agents and precipitation inhibitors: a case study. Mol Pharm 9(5):1100–1108
Loftsson T, Brewster ME (2010) Pharmaceutical applications of cyclodextrins: basic science and product development. J Pharm Pharmacol 62(11):1607–1621
Loftsson T, Brewster ME (2011) Pharmaceutical applications of cyclodextrins: effects on drug permeation through biological membranes. J Pharm Pharmacol 63(9):1119–1135

Long G, Works J (2013). Innovation in the biopharmaceutical pipeline: a multidimensional view. www.pharma.org

Lorenz W, Reimann HJ et al (1977) Histamine release in dogs by Cremophor E1 and its derivatives: oxethylated oleic acid is the most effective constituent. Agents Actions 7(1):63–67

Lorenz W, Schmal A et al (1982) Histamine release and hypotensive reactions in dogs by solubilizing agents and fatty acids: analysis of various components in cremophor El and development of a compound with reduced toxicity. Agents Actions 12(1–2):64–80

Lui CY, Amidon GL et al (1986) Comparison of gastrointestinal pH in dogs and humans: implications on the use of the beagle dog as a model for oral absorption in humans. J Pharm Sci 75(3):271–274

Lukas G, Brindle SD et al (1971) The route of absorption of intraperitoneally administered compounds. J Pharmacol Exp Ther 178(3):562–564

Luke DR, Tomaszewski K et al (2010) Review of the basic and clinical pharmacology of sulfobutylether-beta-cyclodextrin (SBECD). J Pharm Sci 99(8):3291–3301

M3(R2) Guidance on nonclinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals. I. C. o. Harmonization

Maas J, Kamm W et al (2007) An integrated early formulation strategy—from hit evaluation to preclinical candidate profiling. Eur J Pharm Biopharm 66(1):1–10

Mactier RA, Khanna R et al (1987) Role of peritoneal cavity lymphatic absorption in peritoneal dialysis. Kidney Int 32(2):165–172

Mathias NR, Hussain MA (2010) Non-invasive systemic drug delivery: developability considerations for alternate routes of administration. J Pharm Sci 99(1):1–20

Merisko-Liversidge EM, Liversidge GG (2008) Drug nanoparticles: formulating poorly water-soluble compounds. Toxicol Pathol 36(1):43–48

Miller JM, Dahan A (2012) Predicting the solubility-permeability interplay when using cyclodextrins in solubility-enabling formulations: model validation. Int J Pharm 430(1–2):388–391

Morphy R (2010) Selectively nonselective kinase inhibition: striking the right balance. J Med Chem 53(4):1413–1437

Mu J, Woods J et al (2006) Chronic inhibition of dipeptidyl peptidase-4 with a sitagliptin analog preserves pancreatic beta-cell mass and function in a rodent model of type 2 diabetes. Diabetes 55(6):1695–1704

Mu J, Petrov A et al (2009) Inhibition of DPP-4 with sitagliptin improves glycemic control and restores islet cell mass and function in a rodent model of type 2 diabetes. Eur J Pharmacol 623 (1–3):148–154

Muramatsu RS, Litzinger MH et al (2010) Alternative formulations, delivery methods, and administration options for psychotropic medications in elderly patients with behavioral and psychological symptoms of dementia. Am J Geriatr Pharmacother 8(2):98–114

Musther H, Olivares-Morales A et al (2013) Animal versus human oral drug bioavailability: do they correlate? Eur J Pharm Sci 57:280–291

Nagapudi KJ, Jona J (2008) Amorphous active pharmaceutical ingredients in preclinical studies: preparation, characterization, and formulation. Curr Bioactive Compd 4(4):213–224

Nagilla R, Nord M et al (2011) Cassette dosing for pharmacokinetic screening in drug discovery: comparison of clearance, volume of distribution, half-life, mean residence time, and oral bioavailability obtained by cassette and discrete dosing in rats. J Pharm Sci 100(9):3862–3874

Neely BJ, Madihally SV et al (2009) Nonlinear quantitative structure-property relationship modeling of skin permeation coefficient. J Pharm Sci 98(11):4069–4084

Neervannan S (2006) Preclinical formulations for discovery and toxicology: physicochemical challenges. Expert Opin Drug Metab Toxicol 2(5):715–731

Oh DM, Curl RL et al (1993) Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: a mathematical model. Pharm Res 10(2):264–270

Pammolli F, Magazzini L et al (2011) The productivity crisis in pharmaceutical R&D. Nat Rev Drug Discov 10(6):428–438

Paudel KS, Milewski M et al (2010) Challenges and opportunities in dermal/transdermal delivery. Ther Deliv 1(1):109–131
Pfeil E, Dobs AS (2008) Current and future testosterone delivery systems for treatment of the hypogonadal male. Expert Opin Drug Deliv 5(4):471–481
Pole DL (2008) Physical and biological considerations for the use of nonaqueous solvents in oral bioavailability enhancement. J Pharm Sci 97(3):1071–1088
Porter CJ, Pouton CW et al (2008) Enhancing intestinal drug solubilisation using lipid-based delivery systems. Adv Drug Deliv Rev 60(6):673–691
Pouton CW, Porter CJ (2008) Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. Adv Drug Deliv Rev 60(6):625–637
Qian F, Huang J et al (2010) Drug-polymer solubility and miscibility: stability consideration and practical challenges in amorphous solid dispersion development. J Pharm Sci 99(7):2941–2947
Rabinow BE (2004) Nanosuspensions in drug delivery. Nat Rev Drug Discov 3(9):785–796
Rao VM, Stella VJ (2003) When can cyclodextrins be considered for solubilization purposes? J Pharm Sci 92(5):927–932
Reed KW, Yalkowsky SH (1985) Lysis of human red blood cells in the presence of various cosolvents. J Parenter Sci Technol 39(2):64–69
Roth BL, Sheffler DJ et al (2004) Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. Nat Rev Drug Discov 3(4):353–359
Saxena V, Panicucci R et al (2009) Developability assessment in pharmaceutical industry: an integrated group approach for selecting developable candidates. J Pharm Sci 98(6):1962–1979
Scannell JW, Blanckley A et al (2012) Diagnosing the decline in pharmaceutical R&D efficiency. Nat Rev Drug Discov 11(3):191–200
Shah AK, Agnihotri SA (2011) Recent advances and novel strategies in pre-clinical formulation development: an overview. J Control Release 156(3):281–296
Sheth AR (2011) In vitro screening methods to assess the potential of in vivo precipitation of injectable formulations upon intravenous administration. PDA J Pharm Sci Technol 65 (1):71–80
Shiotani K, Uehata K et al (1995) Differential effects of sulfate and sulfobutyl ether of beta-cyclodextrin on erythrocyte membranes in vitro. Pharm Res 12(1):78–84
Six K, Verreck G et al (2004) Increased physical stability and improved dissolution properties of itraconazole, a class II drug, by solid dispersions that combine fast- and slow-dissolving polymers. J Pharm Sci 93(1):124–131
Stella VJ, He Q (2008) Cyclodextrins. Toxicol Pathol 36(1):30–42
Stella VJ, Rao VM et al (1999) Mechanisms of drug release from cyclodextrin complexes. Adv Drug Deliv Rev 36(1):3–16
Strickley RG (2008) Formulation in drug discovery (chapter 24). Annu Rep Med Chem 43:419–451
Sutton SC, Engle K et al (1993) Intranasal delivery of the bisphosphonate alendronate in the rat and dog. Pharm Res 10(6):924–926
ten Tije AJ, Verweij J et al (2003) Pharmacological effects of formulation vehicles: implications for cancer chemotherapy. Clin Pharmacokinet 42(7):665–685
Tong WQ, Whitesell G (1998) In situ salt screening—a useful technique for discovery support and preformulation studies. Pharm Dev Technol 3(2):215–223
Traber MG, Kayden HJ et al (1986) Absorption of water-miscible forms of vitamin E in a patient with cholestasis and in thoracic duct-cannulated rats. Am J Clin Nutr 44(6):914–923
Turner PV, Brabb T et al (2011) Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci 50(5):600–613
Van Eerdenbrugh B, Van den Mooter G et al (2008) Top-down production of drug nanocrystals: nanosuspension stabilization, miniaturization and transformation into solid products. Int J Pharm 364(1):64–75
Vandecruys R, Peeters J et al (2007) Use of a screening method to determine excipients which optimize the extent and stability of supersaturated drug solutions and application of this system to solid formulation design. Int J Pharm 342(1–2):168–175

Vasconcelos T, Sarmento B, Costa P (2007) Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. Drug Discov Today 12(23–24):8

Venkatesh S, Lipper RA (2000) Role of the development scientist in compound lead selection and optimization. J Pharm Sci 89(2):145–154

Verreck G, Six K et al (2003) Characterization of solid dispersions of itraconazole and hydroxypropylmethylcellulose prepared by melt extrusion—part I. Int J Pharm 251 (1–2):165–174

Vitic Nexus. Lhasa Limited, Leeds, UK. http://www.lhasalimited.org/products/vitic-nexus.html

Vo CL, Park C et al (2013) Current trends and future perspectives of solid dispersions containing poorly water-soluble drugs. Eur J Pharm Biopharm 85(3):799–813

Warren DB, Benameur H et al (2010) Using polymeric precipitation inhibitors to improve the absorption of poorly water-soluble drugs: a mechanistic basis for utility. J Drug Target 18 (10):704–731

Waters C (2013) The development of the rotigotine transdermal patch: a historical perspective. Neurol Clin 31(3 Suppl):S37–S50

Watkinson AC (2013) A commentary on transdermal drug delivery systems in clinical trials. J Pharm Sci 102(9):3082–3088

Weiss RB, Donehower RC et al (1990) Hypersensitivity reactions from taxol. J Clin Oncol 8 (7):1263–1268

Williams HD, Trevaskis NL et al (2013) Strategies to address low drug solubility in discovery and development. Pharmacol Rev 65(1):315–499

Willmann S, Schmitt W et al (2004) A physiological model for the estimation of the fraction dose absorbed in humans. J Med Chem 47(16):4022–4031

Woodburn K, Kessel D (1994) The alteration of plasma lipoproteins by cremophor EL. J Photochem Photobiol B 22(3):197–201

Wright BA, Waters CH (2013) Continuous dopaminergic delivery to minimize motor complications in Parkinson's disease. Expert Rev Neurother 13(6):719–729

Xu S, Dai WG (2013) Drug precipitation inhibitors in supersaturable formulations. Int J Pharm 453 (1):36–43

Yalkowsky SH (1999) Solubility and solubilization in aqueous media. American Chemical Society, New York

Yamada I, Haga K (1990) Measurement of gastric pH during digestion of a solid meal in dogs. Chem Pharm Bull (Tokyo) 38(6):1755–1756

Yin AY, Htun M et al (2012) Reexamination of pharmacokinetics of oral testosterone undecanoate in hypogonadal men with a new self-emulsifying formulation. J Androl 33(2):190–201

Zhang H, Zhang J et al (2002) Oral mucosal drug delivery: clinical pharmacokinetics and therapeutic applications. Clin Pharmacokinet 41(9):661–680

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