Application of formulation technologies in lead candidate selection and optimization

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Formulation development during early drug discovery and lead optimization, involves several challenges including limited drug supply, the need for rapid turnaround, and limited development time. It is also desirable to develop initial formulations that will be representative of final commercial formulations. Nanoparticles offer a unique platform for the formulation of poorly soluble drugs – such formulations can be injected (intravenous, subcutaneous, intramuscular), as well as administered through other routes, such as oral, ocular and inhalation. Thus, a single formulation can be used to test and eventually develop multiple dosage forms. Furthermore, nanoparticles offer the opportunity for high drug loading, for low potency compounds, and thus support toxicological evaluation of such compounds.

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▼ The process of taking a new drug from concept to clinic and eventually to commercialization involves several steps that traditionally occur in series. The average cost of taking a compound from discovery stage to commercialization is estimated to exceed US$800 million, with an average development time span of ~15 years, and a success rate of only one in 5000 discovery compounds reaching the market [1]. Given these alarming statistics, pharmaceutical companies are under constant pressure to streamline the drug development process [2,3]. The recent spate of drug patent expirations and subsequent generic competition for blockbuster drugs has further aggravated the need for efficient strategies to screen, identify and optimize lead compounds for development.

High throughput screening (HTS) and combinatorial chemistry methodologies have been developed in the past two decades, to synthesize a vast number of compounds using limited resources. Several complementary *in silico* and *in vitro* strategies have also emerged to screen these compounds and assess their potential to become lead candidates. Compounds emerging as 'hits' from these screening processes are characterized further and tested *in vivo* for safety and efficacy. This phase of preclinical drug development requires that the compound be formulated into a dosage form that can be used to administer the drug. Although simple conventional formulation technologies are preferred at this stage, such technologies are often not applicable for challenging compounds, such as those exhibiting poor aqueous solubility. Combinatorial chemistry and HTS strategies have been reviewed in multiple publications [4–6]. However, literature on rapid formulation development methodologies to develop effective dosage forms for animal testing has been sparse.

Rapid formulation screening and identification of an appropriate formulation are crucial for an accurate assessment of compounds in a drug discovery setting. The challenges involved with formulation during this stage are, the limited availability of the compound, limited drug characterization data, and a need for rapid turnaround. Given these challenges, screening technologies are required to be versatile, robust and scalable. Two approaches can be taken to formulate compounds in this stage of discovery: (a) rapid formulation development, wherein compounds are formulated rapidly for *in vivo* testing, not necessarily considering the commercial viability of the formulation (i.e. the powder in a bottle approach); and (b) robust and thorough formulation development, wherein effort is made to make the formulation commercially

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viable. The rapid formulation development is desirable in situations involving screening of multiple candidates with a limited assigned probability of success. A more detailed formulation development is desired early on, especially in situations involving time constraints and for 'leads' with higher probability of success. There is a further desire to converge these two approaches, by the development and use of rapid formulation techniques that are scalable and commercially viable [7]. This article focuses on the commercially viable enabling technologies that are available to a formulation scientist during drug discovery and lead optimization stages. Although principles of lead optimization are valid for both small molecules and biopharmaceuticals, the focus of this paper is on small molecular weight drugs (Mw < 1000 Daltons).

The drug discovery process

Drug discovery has evolved from rational drug design, which was slow and time consuming, to HTS and combinatorial chemistry technologies that can result in up to 10^4 compounds synthesized per chemist per year [8]. Drug discovery productivity has been accelerated further by the availability of information from the genomic database, which provides new biological targets for drug development. Under such circumstances the appropriate screening of the vast number of compounds becomes crucial for the success of the drug discovery program.

A Biopharmaceutical Classification System (BCS) has been developed to categorize hit compounds, based on their aqueous solubility and gastrointestinal permeability [9]. Compounds with high permeability and high solubility (classified as BCS Class I) are generally easiest to formulate – a simple powder for reconstitution is sufficient for early studies [6]. However, poorly soluble and/or poorly permeable compounds pose a significant challenge. Such compounds often require an array of technologies to provide a viable formulation. Early screening does not differentiate compounds based on their solubility, therefore, there is an increasing bias towards lipophilic (and consequently poorly soluble) drugs entering and progressing through the drug development pipeline. Furthermore, retrospective analysis suggests that drug discovery methodologies are biased towards low potency compounds, with a typical potency in the range of 1mg kg^{-1} [10].

Formulation tools for drug development

Two of the most important preformulation factors that are considered during compound screening are solubility and permeability [11]. Both these factors directly affect the oral bioavailability of the compound. Solubility of the drugs is estimated *in silico* based on structure and can be experimentally verified using techniques such as Laser Nephelometry [12]. Similarly, permeability can be estimated from several semi-empirical models [such as those based on quantitative-SARs (QSAR)] [13,14]. Additionally, software programs are now available to estimate intestinal absorption [15]. Compounds that are identified as poorly soluble and/or show poor bioavailability might require additional formulation expertise at the development stage, as discussed in subsequent sections. However, irrational decisions based on these factors can lead to elimination of compounds that might otherwise be promising.

Conventional formulation technologies

pH adjustment and salt selection are the two most conventional approaches to formulate poorly soluble drugs. Ware and Lu discuss an automated approach to salt selection – a 96-well plate was used to form salts of a drug, trazadone [16]. The salts were evaluated under microscope to assess their crystallinity. Crystalline salts were further scaled-up and characterized using differential scanning calorimetry (DSC), powder X-ray diffraction (PXRD), hygroscopic, pH-solubility, density, surface area and particle size analyses. A tosylate salt was identified as a promising candidate for further evaluation. This case study provides an example of identification of a less common salt of an insoluble drug, leading to a pharmaceutically viable and promising formulation. Perng and coworkers developed a simple flow through cell based dissolution assay, as a tool for salt and formulation screening in an early discovery setting [17]. This apparatus was used for rapid screening of various formulations and salts for a poorly soluble drug. A good correlation was observed between results of the flow-through cell apparatus and *in vivo* oral bioavailability for multiple formulations. Another case study describing formulation development for a poorly soluble discovery compound has been presented by Krishna and coworkers [18]. A targeted 5–10 mg ml^{-1} solubility was achieved for this compound (with intrinsic solubility ~100 μg ml^{-1}), by use of a 100% cosolvent system, consisting of 70% polyethylene glycol 300 and 30% ethanol, with citric acid added for acidification and to prevent precipitation

Lee *et al.* presented a decision tree to develop formulations for challenging drugs for intravenous administration [19]. Their methodology involved the use of pH adjustment and cosolvents to solubilize compounds for early screening. The combination of these two approaches leads to the following equation:

$$S_{eff} = S_{HA}\,[10^{\acute{o}_{HA} f_{cosol}} + (10^{(pH-pKa)} . 10^{\acute{o}_{A} - f_{cosol}})] \qquad \text{[Eqn 1]}$$

where S_{eff} is the effective aqueous solubility, S_{HA} is the weak acid (or base) solubility, σ_{HA} and σ_A are the solubility powers

of the cosolvent for the neutral and ionized forms, and f_{cosol} is the fraction of cosolvent in the system. This equation could be used to estimate the effective solubility of a drug, wherein both pH and cosolvent are used synergistically to solubilize the drug. The combined pH-cosolvent approach was applied to more than 300 discovery compounds. It was observed that 11% of compounds were not formulatable using this approach, and another 32% of the formulations used >55% cosolvent [19]. The latter aspect (high solvent ratio), can limit the pertinent safety assessment of lead compounds, especially during long-term exposure studies where the drug is required to be dosed at several multiples of the projected human doses. This suggests that conventional technologies or even synergistic combinations of the same might not be sufficient to develop commercially viable formulations for poorly soluble drugs. This has led to the incorporation of drug delivery technologies into early discovery stages [20].

Enabling formulation technologies

Novel enabling technologies are desired for challenging drugs where conventional technologies do not provide a viable solution. Through drug delivery research, several enabling technologies are now becoming available to formulate compounds that are poorly soluble. At the same time, formulation scientists can not merely depend on these technologies to assist development at a later stage – the use of these technologies and subsequent experimental demonstration of this are crucial elements required in lead optimization. Hence there is a need for such enabling technologies to be tested at small scales in a drug discovery setting.

Solid dispersions

Solid dispersion technologies involve stabilization of the drug in its amorphous form, within a carrier matrix. The amorphous form allows faster dissolution of the drug and is particularly promising for orally administered drugs (because of the wider choices of carrier matrices). Typical carriers used for this technology include water-soluble polymers, such as polyvinylpyrrolidone, polyethylene glycol and so on. This technology was first reported more than three decades ago, however, its use was limited due to several manufacturing challenges. Several recent advances, including better solvent removal techniques, better understanding of the amorphous stabilization, and manufacturing advances have led to a revived interest in this technology for poorly soluble drugs [21]. However, to use this technology effectively identification of an appropriate carrier that is compatible with the drug is necessary. Lee *et al.* described a rapid screening technique to study the miscibility of drugs with carriers towards preparation of solid dispersion formulations [22]. The drug and carrier are dissolved in a common solvent and 2–3 drops are placed on the center of a silicon chip. The chip is spun at high speeds to rapidly evaporate the solvent, leaving behind a dispersion film of drug and carrier on the chip. The film is studied under an optical microscope to examine crystalline domains or amorphous patterns that would indicate the morphology of the drug in a solid dispersion matrix. This technique can be used to screen different carriers and different drug:carrier ratios to optimize a solid dispersion formulation with minimal use of the drug. The drug–carrier screening tool can be used in conjunction with miniaturized processing equipment (such as the Micro-Spray Dryers developed by Particle and Coating Technologies; http://www.pctincusa.com), to develop and screen solid dispersion formulations of poorly soluble drugs. Although solid dispersions are limited to the oral route of administration, the concept of carriers and excipient screening can also be broadly adopted to other technologies.

Solubilizing agents

Complexation using carriers such as cyclodextrins is another approach that can be used for solubilization and screening of poorly soluble compounds [23]. Cyclodextrins solubilize molecules through the formation of an inclusion complex. Cyclodextrin complexation might at times provide unique pharmacological properties to a drug. It was demonstrated by Athanasiou and coworkers that the antimicrobial activity, in terms of minimum inhibitory concentration, of β lactam antibiotics–cyclodextrin complexes was better than the drug alone. This effect was believed to be more than solubility induced [24]. Nakamura and coworkers described a case where several buffers were screened to solubilize a discovery compound YM466, and achieved a target concentration of 5 mg ml^{-1}. The addition of cyclodextrins, specifically β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin and γ-cyclodextrin, resulted in significant enhancement in solubility, up to 7–8 mg ml^{-1} [25].

Despite the significant promise of cyclodextrins, these solubilizing aids have their own limitations. Several naturally occurring and commercially available cyclodextrins have exhibited toxicity, especially at higher concentrations [26], and can impede intravenous toxicology studies of the drug. Furthermore, the use of cyclodextrins in a formulation can limit the dose level, depending on the toxicity and solubilization potential of the carrier. Finally, cyclodextrins require the drug to have specific molecular properties to efficiently form a complex. Rao and Stella

have provided some guidelines on the use of cyclodextrins for complexation [27]. A dimensionless number U(CD), (termed the cyclodextrin utility number), was introduced to assess the feasibility of the use of CDs in dosage forms. U(CD) is a function of the dose of the drug, the workable amount of CD, the binding constant, and the drug solubility in the absence of CDs. When U(CD) is ≥1 for a drug–cyclodextrin system, adequate solubilization of the drug in cyclodextrin can be expected. Thus, an initial feasibility on the use of cyclodextrins can be assessed before actual experimentation work begins [27].

Other common solubilization agents include Tween 80 and PEG400, however, as in the case with cyclodextrins, high amounts of solubilizing agents might lead to excipient-related dose limitations [28]. High amounts can also cause interference during analytical testing of drug candidates [29] and result in false rejection of compounds during discovery screen. To overcome some of the analytical issues associated with common solubilizers, Temesi *et al.* developed a monodisperse PEG_{414} vehicle that provides the same solubilizing effect as PEG_{400}, and has a single peak as opposed to multiple peaks associated with PEG_{400} [30].

Lipid-based complexation

Complexation using lipids is another strategy that can be adopted for poorly soluble drugs. Various complexes can be formed including liposomes, micelles, cubosomes and solid lipid nanoparticles (for reviews see [31,32]). Lipids can also be used in developing self-emulsifying drug delivery systems (SEDDS). Lipid vesicles, particularly liposomes, have been used extensively for targeting of chemotherapeutic drugs to tumors [33]. Liposomes provide an additional benefit of being applicable for both water soluble drugs (whereby the drug is encapsulated in the aqueous core of the vesicle) and water insoluble drugs (whereby the drug is trapped in the lipid bilayers). Finally, for oral applications, lipid vesicles might serve as permeation enhancers, increasing the bioavailability of drugs [32].

Although most of lipid complex-based technologies provide a potential to formulate challenging drugs, they suffer from the drawback that overloading of the drug could lead to destabilization of the system. This can limit the use of this technology only to high potency drugs. Furthermore, the presence of high amounts of lipids might provide an analytical challenge in identifying the effect of the drug versus the excipient.

Particle size reduction

Poorly soluble drugs can be formulated as nanoparticles with high surface area, which enhances dissolution rates in accordance with the Noyes-Whitney equation:

$$\frac{dQ}{dt} = \frac{D}{h} S\,(C_s - C_g) \qquad \text{[Eqn 2]}$$

where the rate of dissolution (dQ/dt) is directly proportional to the diffusion coefficient of the drug (D), the available surface area (S), and the difference between saturation solubility of the drug in the boundary layer (C_s) and concentration of drug in the bulk fluid (C_g).

Particle size reduction can be achieved mainly by two types of processes: (1) Particle formation processes (microprecipitation, chemical synthesis, complexation), and (2) Particle comminution (size reduction) processes (homogenization, milling, grinding) [34]. The two types of processes can also be combined for a synergistic effect as adopted by the NANOEDGE technology platform (Baxter Healthcare; http://www.baxterdrugdelivery/com) [35]. A particle formation step (microprecipitation) is employed just before a particle comminution step. In this platform, a particle formation step is designed to produce customized, friable particles that are amenable to comminution via high-pressure homogenization [35,36].

Besides enhanced dissolution, nanoparticles offer the potential for passive or active targeting of drugs [33]. When administered intravenously, nanoparticles have a tendency to be taken up by the reticuloendothelial system (RES). This provides a potential for targeting for macrophage-related disorders. Nanoparticles can be coated with hydrophilic polymers, such as polyethylene glycol, to prevent RES uptake. Such particles have a tendency to circulate for longer periods of time, and are typically taken up by tumor cells via the enhanced permeation and retention (EPR) effect. Furthermore, nanoparticles can be coated with specific functional groups for active targeting. For example, thiamine coated nanoparticles demonstrated preferential uptake in the brain, via the blood–brain–barrier thiamine transporters [37]. Nanoparticles can also be administered via multiple routes of administration, as a solid or a suspension. Thus, a single formulation can lead to multiple dosing options early in the development program. Given these features, nanoparticles have found wide use in recent years for various oral [38], injectable [35,39,40], inhalational [41] and even intradermal [42] applications. In each case, the formulation can provide a potential benefit, as listed in Table 1. Figure 1 depicts a flowchart of activities that can be followed for routine testing of compounds for lead selection, using nanosuspension technology.

Nanoparticles further provide clinical benefits including enhanced bioavailability, reduced fed-fasted ratio (for oral applications). Given these factors it is anticipated that nanoparticles could offer potential solution for expediting the lead selection and the drug development process.

It should be noted that given the intense energy used in particle comminution processes, nanoparticulate formulations typically require the drug to have a melting point of >50°C. An analysis of 317 discovery compounds reported by Lee *et al.* indicates that most of the compounds emerging from the discovery stage satisfy this requirement [19]. It should be further noted that depending on the dissolution rates, nanoparticles might have varying pharmacokinetic impact [20]. Rapidly dissolving formulations act as solutions, while slow dissolving formulations can be used for targeting applications.

Table 1. Potential benefits of nanoparticles over conventional formulation technologies, in multiple routes of administration, for poorly soluble drugs

Route of administration	Potential benefit
Oral	Rapid onset Reduced fed-fasted ratio Improved bioavailability
Intravenous	Rapid dissolution Tissue targeting
Subcutaneous/Intramuscular	Rapid onset Reduced tissue irritation Higher bioavailability
Inhalation	Higher bioavailability More consistent dosing
Ocular	Higher bioavailability More consistent dosing

Rapid formulation screening

Several platforms have been developed to provide rapid formulation screening. These platforms involve a combination of one or more traditional and novel enabling technologies. One such rapid formulation screening technology has been discussed by Chen and coworkers [43]. A high throughput tool was employed to screen 9880 formulations, to identify a potent cremophor-free formulation of paclitaxel. Such a formulation can be administered in higher amounts, given the elimination of the dose-limiting excipient. The previously discussed nanoparticulate technologies (e.g. NANOEDGE) have also been fitted with rapid screening modality for drug discovery support.

The applicability of rapid screening nanoparticulate platform has been proposed in Figure 1 for lead product selection. Poorly soluble drugs (solubility less than 500 ppm) are processed rapidly (and in small quantities) as nanosuspensions that are stabilized either in suspension or frozen or lyophilized. The suspension is tested in animals for efficacy and the process is repeated iteratively till the 'hit' compound(s) with desired efficacy is identified. By providing the capability for high doses, preliminary toxicology data can also be generated at this stage. The formulation can then be optimized based on the desired route of administration. Comparison with intravenous administration can be used to determine bioavailability (for rapidly dissolving formulations), when extravascular route of administration is pursued. Nanoparticles have been used in all various routes, therefore, formulation optimization at this stage might simply involve choosing the appropriate excipients for that route

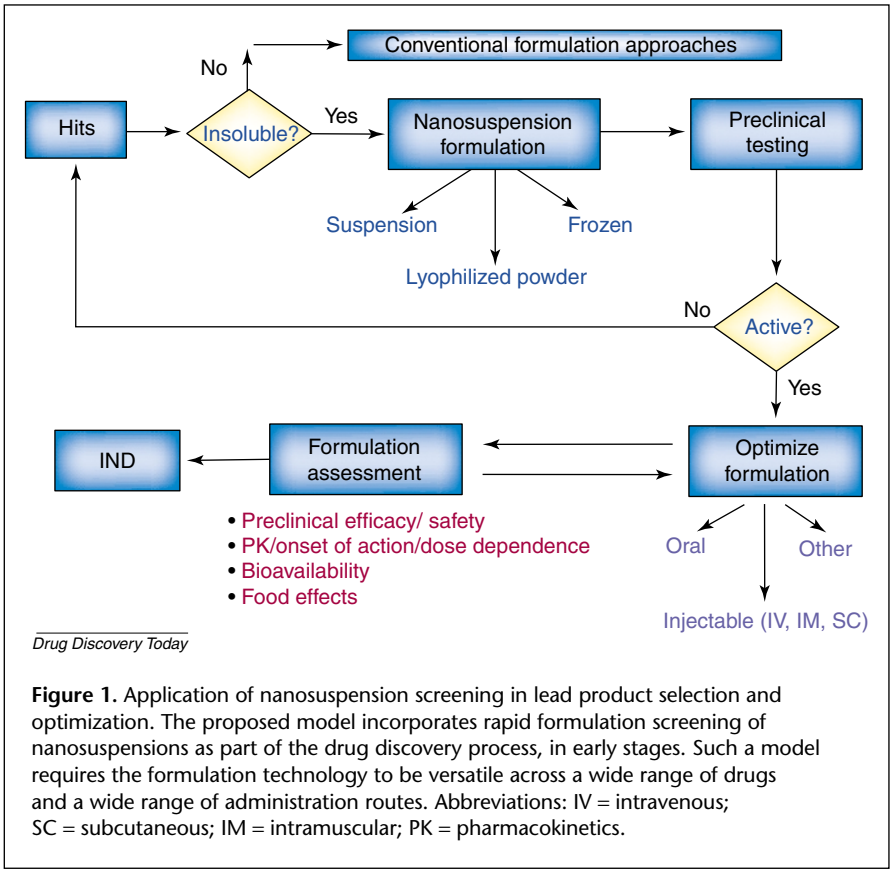

Figure 1. Application of nanosuspension screening in lead product selection and optimization. The proposed model incorporates rapid formulation screening of nanosuspensions as part of the drug discovery process, in early stages. Such a model requires the formulation technology to be versatile across a wide range of drugs and a wide range of administration routes. Abbreviations: IV = intravenous; SC = subcutaneous; IM = intramuscular; PK = pharmacokinetics.

Table 2. Comparison of different drug delivery technologies for drug discovery support.

Technology	High Dose?	Mp <40	Route			Rapid screening?
			Injectable	Inhalation	Oral	
Nanoparticles	●		●	●	●	●
Liposomes		●	●	●		
Lipid complex		●	●		●	
Cyclodextrins		●	●			●
Emulsions		●	●		●	●

of administration. More rigorous testing can be performed at this stage to assess the formulation before scale-up. It is anticipated that other versatile technologies with rapid screening functionalities might also be used in a similar fashion.

Discussion

Formulation development during early drug discovery and lead optimization involves a balance between rapid screening and commercially viable formulation development. Poorly soluble drugs pose an additional challenge in terms of development of a formulation with acceptable bioavailability (for oral/extravascular applications), and acceptable safety and toxicology for intravenous administration. This challenge is further aggravated for low potency drugs, given the high dose requirements. Drug delivery technologies have been developed to address these issues, and several of these have been scaled down to meet the needs of drug discovery support. The choice of the appropriate technology depends strongly on factors such as proposed indication, projected dose, and route of administration. Table 2 provides a comparison of various technologies for specific decision factors. Scalability of the technology and long-term stability of the formulation should also be considered in the decision-making. Given this complexity of selection, and the fact that several of the decisive factors might not be known in early discovery, versatile technologies that can be used for multiple routes of administration are especially desirable. It should be noted that each technology has a significant and unique impact on the drug pharmacokinetics [20]. Furthermore, a single technology can be manipulated to produce different pharmacokinetics profiles. These factors point to the need for involving pharmaceutical scientists equipped with drug delivery tools, early in the drug discovery and development cycle. Recent reports from various early formulation laboratories [3,7,20] affirm a trend towards adoption of this strategy within the pharmaceutical industry.

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