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Review

Developing early formulations: Practice and perspective☆

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Abstract

Early formulations are prepared mostly for drug compounds at both discovery and preclinical stages and are used to animals via various routes such as oral and intravenous dosing. They serve the purpose of evaluating these compounds on a broad range of pharmaceutical interests, notably pharmacology (activity/efficacy), pharmacokinetics (PK), and toxicology. It is estimated that approx. 40% of all drug compounds discovered have certain delivery limitations due to poor solubility or poor bioavailability. This brings tremendous challenges to the scientists working in the field of early formulations.

This study intends to cover a broad spectrum of early formulations including basic aspect and development aspect. On basic aspect, it summarized early formulation study purpose, objectives, dosing route, animal species, etc. It then evaluated a variety of dosage forms and solubility enhancement approaches including various solutions, suspensions, lipid-based formulations, solid dispersions, etc. On development aspect, this study broadly reviewed literatures and current practice in the field, the issues and challenges. It offered authors' own approaches and strategies including general development schemes for oral and for i.v., recommended excipient use range for oral and for i.v., experimental procedures for *vitro* serial dilution method, for kinetic solubility, etc. The study also discussed a number of case analyses and emphasized scientific rationales and experimental approaches in each of them. The study concluded with authors' summary and some comments on early formulation practice, thoughts and perspectives on its future trend.

The study is a mixture of literature review and investigational research. It provides many useful information, practical procedures, and recommendations. It is expected that the study will fill the void of literature of such kind, and provide direct benefit to everyday practitioners in the field.

Keywords: Early formulations; Solubility enhancement; Poorly water-soluble drugs; Animal formulations

Contents

☆ Views and suggestions appeared in this article represent those of the authors, and not the affiliated organizations.

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1. Introduction

Early formulation development is an integral part in drug discovery and development. These formulations are prepared mostly for drug compounds at both discovery stage (discovery lead) and preclinical stages (preclinical lead) and are dosed to animals via various routes such as oral and intravenous. Hence, early formulations are also known as animal formulations or preclinical formulations. A primary purpose for these formulations is to acquire sufficient *in vivo* exposure in order to select, optimize and advance compounds on a broad range of pharmaceutically related properties/profiles including pharmacology, pharmacokinetics (PK), and toxicology.

Listed below are a few important aspects/objectives in developing and characterizing an early formulation:

(a) *Optimum in vivo exposure at the target site*. This is a prerequisite to evaluating biological or pharmacological response.
(b) *Accurate dosing*. This is a prerequisite to establishing dose–response relationship, which requires both chemical and physical stability of the formulation throughout preparation, dosing as well as short-term storage.
(c) *Minimized excipient-related side-effect or toxicity*. This is self-evident: an vehicle or formulation needs to maintain maximum biocompatibility and *in vivo* tolerability.

2. Background

2.1. Study purpose

It is important to understand the broad scope of animal studies in drug compound evaluation, the objective, the process of the experimentation, and how early formulation can support and improve the study outcome. In general there are three major categories in animal studies.

2.1.1. Pharmacology study

Pharmacology study is to evaluate interaction between a drug compound and biological, pharmacological, or physiological effect(s), and to evaluate what a drug does to the body, the mechanism of its action, and the correlation between the drug concentration and the effect. A variety of studies are

under this category: activity/efficacy ranking, disease model development, extensive activity/efficacy studies with respect to specificity/selectivity/tolerability, dose-related activity/efficacy in validated disease models with proposed clinical route, activity/efficacy comparison with competitor's drug compounds, etc.

Both solutions and suspensions are often used in the study. It is generally required that vehicles for the study should be well tolerated.

2.1.2. Pharmacokinetics study

Pharmacokinetics (or PK) study is to evaluate the absorption, distribution, metabolism and excretion (ADME) of drugs in human and animals. These properties are closely related to drug pharmacological and toxicological actions. Early formulations are broadly involved throughout a typical PK profiling in support of IND (investigational new drug) filing: single dose PK, dose proportionality, absolute bioavailability, multiple dose PK, etc. More extensive PK evaluation may involve food effects, tissue distribution, brain penetration, etc. The PK study may be conducted in rodent and non-rodent species via various routes of administration.

The PK study probably constitutes the main workload from customers' request. The experimental outcome provides a basis for selecting and optimizing drug candidates. In early PK study, solution formulation is often used to measure the exposure and identify issues with absorption mechanism: the drug is either dissolution or permeability rate-limited. Solution is a well-defined physical state: there is no dissolution process, so it excludes variation from solid state. Suspension is also widely used both in early PK and late PK. It provides more of a 'reality check' for future oral solid dosage development, a default dosage form for most of the drug compounds.

2.1.3. General toxicology study

General toxicology (or tox) study is to evaluate the adverse effects of drug compounds *in vivo* in order to develop a safe clinical dose range. A toxicology package for an IND filing may include acute dose, or dose ranging finding or maximum tolerated dose (MTD), and a 14- or 28-day repeated dose toxicity study with or without recovery period, conducted in one rodent and one non-rodent species. An NDA (new drug application) filing usually require longer-term repeated dose studies and may require reproductive and/or carcinogenicity studies depending on the specific drug application.

In many situations, formulations for toxicology studies present significant challenges due to the fact that these formulations require high concentrations and need to be used over a long period of time. For example, in dose escalating studies, a common scenario is that the formulation is required to deliver a multiple folds greater *in vivo* exposure than the efficacious concentration. Frequently the formulation is required to dose continuously for certain time period (e.g.: 2 weeks), while at all time the vehicle is expected not to elicit adverse effects. It is worth noting that a toxicology study is often on critical pathway for the lead to progress. The expectation is high: the formulation needs to be prepared with high quality to ensure reliable experiment and data interpretation.

2.2. Animal species, dose

Common animal species include rodents such as mice, rats, guinea pigs, and non-rodents such as dogs, rabbits, sheep, pigs, monkeys. Different species respond differently to the same dose per weight (mg/kg) due to varying physiological and biological conditions. Also, different species may have significantly different metabolic profiles which directly impacts the PK read-out: a drug can have good exposure in one species (e.g.: rats) while having poor one in the other (e.g.: dogs).

Dose is another important factor in seeking maximum exposure in animals: there is a limit as how much one can dose without significantly impacting animal's well being. Table 1 provides a set of useful parameters for a few common species. It lists recommended or normal dosing ranges for oral and intravenous administration. This provides a perspective on early formulations, though in practice the dose often goes beyond the range.

2.3. Dosing route

Common dosing routes include oral, intravenous (i.v.), subcutaneous (s.c.), intra-peritoneal (i.p.), intra-muscular (i.m.), intra-duodenal (i.d.), intra-nasal (i.n.), intra-articular (i.a.), intratracheal (i.t.), ocular, intra-dermal (i.d.), etc.

2.3.1. Oral

Oral is the most common route in animal dosing. Solutions (preferably within pH 2–9), neat cosolvents, suspensions, capsules, (mini)-tablets, or even oils can all be dosed via oral. Gavage, or force feeding, is often used dosing solutions or suspensions in rodent or non-rodent species. This is to introduce the formulations directly into the stomach via a gavage needle or a tube.

Table 1
Relevant physiological parameters for different animals

Animal species	Weight (kg)	Blood volume (ml)	Blood flow (ml/min)	Total surface area (m^2)	Life-span (year)	Normal oral (ml/kg)	Normal i.v. (ml/kg)
Mouse	0.02	1.7	Liver: 1.8; kidney: 1.3	0.008	2.7	10	5
Rat	0.25	13.5	Liver: 13.8; kidney: 9.2	0.023	4.7	5–10	2.5
Rabbit	2.5	165	Liver: 177; kidney: 80	0.17	8.0	5–10	1–2
Rhesus monkey	5	367	Liver: 218; kidney: 138	0.32	22	5–8	0.5–1
Dog	10	900	Liver: 309; kidney: 216	0.51	20	5–8	0.5–1

Modified from Davies and Morris (1993).

Differences in formulations can impact drug dissolution, solubilization and absorption to various degrees. Solution formulation is generally preferred in all animal studies (efficacy, PK, toxicology) due to the dose accuracy, but solution formulation often has limitations with drug concentration and potential drug precipitation (with cosolvents and/or pH buffers). Suspension, on the other hand, is capable of providing high drug concentration, relatively simple preparation procedure. The issue with suspension is that it may risk partial dissolution, as well as batch-to-batch inconsistency.

2.3.2. Intravenous (i.v.)

Solution is generally desired for i.v. (preferably within pH 2–9). In practice, other dosage forms such as micronized- or nano-suspensions, lipid-based formulations are also used, provided that these formulations have well-defined physical properties, and have small and well-defined particle size (preferably in nano-range). For mice and rats, preferred sites for i.v. dosing include tail vein, dorsal penile vein, etc. In recent years, there is an increasing number of i.v. animal formulations using lipid-based system. A few i.v. dosing methods are available: bolus (20–40 s), slow injection (3–8 min), and infusion (varied; e.g.: a couple hours to 24 h). The rate of injection is important in that it minimizes or prevents adverse events (e.g.: cardiovascular failure).

2.3.3. Subcutaneous (s.c.)

If i.v. is a reference point, s.c. can be seen as an i.v. with more restrictions on formulations. Again, solution is the first choice, though a well-defined suspension can be used. For solutions, one needs to pay more attention to the volume to be dosed (much reduced volume), isotonicity, and a narrower pH range (pH 4–8). A physiological pH (close to pH 7.4) is preferred, though not required. Cosolvent(s) can be used, but it is understood that the concentration should be significantly lower than in i.v. The reason is that the cosolvent is hard to diffuse away at the injection site. Suspension is used less frequently and is often reserved for sustained release. Injection site for s.c. dosing is usually in the neck or back where skin tissues are relatively loose.

2.3.4. Intra-muscular (i.m.)

Similarly, if i.v. is a reference point, i.m. can be seen as an i.v. with an increased flexibility on formulations. Both solution (pH 2–9) and suspension can be used. Cosolvents can also be used and with a higher concentrations than i.v. Other formulations that can be used include lipid-based formulations or even oil formulations, but these formulations are mostly for a depot effect. For i.m., drug compounds are rapidly absorbed into the general circulation and lymphatic system.

2.3.5. Intra-peritoneal (i.p.)

Solution-based formulation is preferred because there is little biological fluid in the abdominal (peritoneal) cavity. This site is also sensitive to the animal, so it is advised not to use or to reduce the use of irritating excipients. Recommended vehicles include saline, water or some well-tolerated cosolvents such as propylene glycol or PEG400. If a buffer is used, the pH should be close to physiological pH. The i.p. route provides a fast absorption into general circulation.

2.3.6. Intra-articular (i.a.)

Both solutions and suspensions can be accepted for this local injection route (via joints). Other formulation types may also be used including lipid-based formulations such as emulsions and liposomes.

2.3.7. Intra-tracheal (i.t.)

This route is to dose the formulation into a thin-walled, cartilaginous tube descending from the larynx to the bronchi and carrying air to the lungs. Solution is generally preferred. If a suspension has to be used, the particle size must be reduced via micronization or even nanonization. The study is often carried out in guinea pigs and rats.

2.3.8. Intra-duodenal (i.d.)

This route bypasses the stomach, and goes directly into the duodenal, the beginning portion of the small intestine. Formulations that are acceptable for oral can be accepted for this route.

2.4. Discovery leads versus preclinical leads

Discovery leads refer to those involved in exploratory chemistry, lead selection and optimization. These compounds come in large numbers but small in quantity (e g.: <20–30 mg). They usually are not well characterized, often in amorphous form, and vary in purity from batch to batch. Major interests lie in their activity/efficacy ranking, early PK and early toxicology study outcomes. Solution or suspension is often used as the dosage form.

Preclinical leads (a.k.a. development leads) are generally referred to those emerged from discovery program: they are small in numbers, but large in quantity (e.g.: 50–1000 mg). These compounds usually have fixed chemical structures, though salt selection/polymorph screening is still an on-going process. Major interests include in-depth activity/efficacy, PK and toxicology profiling. Formulation requirement for these compounds is more selective. In addition to solutions and suspensions, novel formulations such as nanosuspensions, solid dispersions, or lipid-based formulations are frequently introduced in various applications (see Section 3).

3. Major dosage forms and solubility enhancement approaches

3.1. pH adjustment

Pharmaceutical buffers are commonly used in solution formulations. The pH buffer works for weak acids or bases that ionize at physiological pH 2–9. Take an example: for a basic drug compound, the total solubility [S_{tot}] is a sum of unionized drug [S_B] and ionized drug [S_{BH^+}], where [S_{BH^+}] can be expressed by Handerson–Hasselbalch equation:

$$[S_{tot}] = [S_B] + [S_{BH^+}] = [S_B] + [S_B] \times 10^{pK_a - pH}$$

Table 2
Product examples—pH control by buffering agents and strong acid/base

	pK_a	pH	Examples of commercial product
Buffering agent			
Maleic acid	1.9, 6.2	2–3	Teniposide
Tartaric acid	2.9, 4.2	2.5–4	Risperidone
Lactic acid	3.8	3–4.5	Ciprofloxacin
Citric acid	3.1, 4.8, 6.4	2.5–7	Loratadine
Acetic acid	4.75	4–6	Mitoxantrone
Sodium bicarbonate	6.3, 10.3	4–9	Cyclophosphamide
Sodium phosphate	2.2, 7.2, 12.4	6–8	Warfarin
Strong acid/base			
Hydrochloric acid	−4	3	Midazolam
Sodium hydroxide	0.2	10–12	Phenytoin

Modified from Lee et al. (2003).

As pH decreases, the drug ionization increases; as a result, the $[S_{tot}]$ increases. The pH at which the drug formulated is determined by both drug solubility and drug solution stability. Hence, it is important to conduct short-term drug solution stability at the formulation pH. Extreme pHs can have biocompatibility issues such as tissue irritation, drug precipitation.

The pH control of the solution formulation is an important component in pharmaceutical products, especially in ophthalmic solutions. Many commercial products have buffer systems with various buffering agents: maleic acid, tartaric acid, glycine, lactic acid, citric acid, acetic acid, etc. (see Table 2). Other products use strong acid (hydrochloric acid) or strong base (sodium hydroxide) to control the solution pH (also see Table 2). Though solution pH control by pharmaceutical buffers or by strong acid/base can be used in various early formulations including oral and i.v. and for various animal species, it is advised that one choose the formulation pH that is close to the pH environment at the targeted dosing site. In general, a range in pH 2–9 is acceptable for most early formulations. Buffer preparation and selection were discussed in many literatures or textbooks. Examples may include Kaus (1998), USP 30/NF 25 (2006), Remington book (Remington: the Science and Practice of Pharmacy, 20th ed., edited by Gennaro, 2000).

It is important to buffer the solution in order to maintain the drug solubility at certain pH. High buffer capacity often reinforces the effect, and helps reduce the drug potential to precipitation (Simamora et al., 1995, 1996) (for precipitation, see more discussion in Sections 3.2 and 4.5). On the other hand, high capacity buffers as well as strong acid/base controlled pH solutions can have negative impact to the overall physiological balance: for a solution formulation into blood stream, it is expected that the buffer system in the formulation do not disrupt the pH/buffer balance in animal's general circulation. Other damaging effect of high buffer capacity and strong pH include tissue irritation/damaging, especially in routes such as, s.c. and i.m. Whenever possible, one needs to optimize the formulation with reduced buffer capacity and with acceptable pH range (pH 2–9), and to inject small volume with extended injection time.

3.2. Use of cosolvents

Cosolvents are known for their solubilizing capacity to most poorly water-soluble drug compounds. Yalkowsky and his colleagues (Yalkowsky and Valvani, 1977, 1980; Yalkowsky, 1999) indicates that, for a water–cosolvent system, there is a semi-logarithmic relationship among total drug solubility $[S_{tot}]$, drug solubility in water $[S_0]$, and the cosolvent volume fraction f as shown below:

$$\log[S_{tot}] = \log[S_0] + \sigma \cdot f$$

The σ is cosolvent solubility power for a given solvent and a given drug. It can be obtained by the slope of the plot on $\log[S_{tot}]$ versus f. The equation shows that the solubility increases as the cosolvent concentration or f increases. The value of σ depends inversely on polarities of both the drug and the cosolvent.

In practice, a mixture of cosolvents is often used in order to reduce vehicle toxicity. A more general equation for cosolvent(s) system is provided as follows where σ_i is solubility power of cosolvent i, and f_i is its volume fraction:

$$\log[S_{tot}] = \log[S_0] + \sum_{i=1}^{n} (\sigma_i \cdot f_i)$$

It was reported that the combined use of cosolvent with pH adjustment can significantly increase drug solubility. While investigating the solubility of drug Flavopiridol, Li et al. (1999c) proposed a model to explain the mechanism of the synergistic solubility enhancement in cosolvent/pH system. For example, for a weak acid drug (HA), the solubility of both unionized and ionized forms in the cosolvent/pH system is described as follows:

$$[S_{tot}] = [S_{HA}^{cosolvent}] + [S_{A^-}^{cosolvent}]$$

$$[S_{HA}^{cosolvent}] = [S_{HA}] \cdot 10^{\sigma_{HA} \cdot f}$$

$$[S_{A^-}^{cosolvent}] = [S_{A^-}] \cdot 10^{\sigma_{A^-} \cdot f} = [S_{HA}] \cdot 10^{(pH - pK_a) + \sigma_{A^-} \cdot f}$$

where σ_{HA} and σ_{A^-} are the solubility power of cosolvent for the unionized and ionized forms, respectively. Hence the $[S_{tot}]$ in a cosolvent/pH system is as follows

$$[S_{tot}] = [S_{HA}] \cdot 10^{\sigma_{HA} \cdot f} + [S_{HA}] \cdot 10^{pH - pK_a + \sigma_{A^-} \cdot f}$$

Common cosolvents include ethanol, PG (propylene glycol), PEG300 (PEG = polyethylene glycol), PEG400, glycerin, *N*,*N*-dimethyl acetamide (DMA), etc. These cosolvents are used with aqueous solutions for oral (as liquids) and for parenteral in animal dosing. Information on cosolvent toxicity data (LD_{50}) with different animal species can be found in many literatures including *Handbook of Pharmaceutical Excipients* (by Rowe, Sheskey and Weller, 4th ed., 2003). Ethanol, PG and PEG are all broadly used in marketed pharmaceutical products (Sweetana and Akers, 1996). For example, there are a number of commercial injection products that contains the same cosolvent composition as 10% ethanol and 40% PG: Valium for Diazepam, Lanoxin for Digoxin, Nembutal for Sodium Pentobarbital, Dilantin for Phenytoin, etc. For animal dosing, PG and PEG300 or PEG400

are used as high as 70–80% both in oral and in parenteral. Dimethylsulfoxide (DMSO) is a powerful solubilizer as well as membrane penetrator, but commercial products by far are only limited to dermal applications (as a penetration enhancer) as well as a few veterinary products. Though DMSO is often used at discovery stage (high-throughput screening), its toxicity and membrane penetration significantly restrict its application in PK and toxicology profiling.

One major limitation using cosolvent(s) is potential drug precipitation once the formulation is administered into the body. This is particularly a concern for i.v. bolus injection where the drug/blood mixing time is limited. The precipitated drug or particulates can have broad implications: pain at injection site, thrombophlebitis (a symptom of vein wall inflammation due to particulate abrasion), erratic blood level, and uneven or delayed bioavailability. Commercial products such as Valium (Diazepam) and Dilantin (Phenytoin) are among reported ones prone to precipitate upon injection/infusion. To minimize or prevent precipitation, it is important to understand the root cause and to conduct certain *in vitro* evaluation on the formulation (see discussion in Section 4.3). A few practical approaches are presented as below:

(a) To apply slow injection (infusion) wherever possible.
(b) To reduce drug concentration in the given cosolvent(s) system.
(c) To incorporate low percentage of surfactant(s) in the formulation.

Precipitation *in vivo* is driven by supersaturation generated from dilution of the cosolvent(s) formulation with aqueous media (e.g.: blood). This is largely a process of fast crystallization. The presence of small amount of surfactants interferes with the formation of crystallization, and as a result, delays, minimizes or even eliminates crystallization process. In a separate research, we investigated a number of poorly water-soluble drug compounds and found that the incorporation of small amount of surfactants (0.05–0.5%, w/v) in cosolvent(s) formulations prevented or minimized drug precipitation from occurring (Zhao, unpublished data). The study shows that, for a given cosolvent formulation, if a surfactant is carefully chosen and used, it can be effective in suppressing or preventing drug precipitation upon injection (see a case study in Section 5.3).

3.3. Use of cyclodextrins

Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity. The α-, β-, and γ-cyclodextrins consist of 6, 7, 8 glucopyranose units, respectively. Naturally occurring cyclodextrins have limited aqueous solubility, while modified cyclodextrins especially those β-cyclodextrin derivatives have shown and delivered the promise to many poorly water-soluble drug compounds. Both HPβCD (hydroxypropyl-β-cyclodextrin) and SBEβCD (sulfobutylether-β-cyclodextrin) stand out as having good solubility and acceptable safety profiles. A large body of literature on these excipients (especially HPβCD, SBEβCD) is available on physical chemical properties, toxicity and safety evaluation, and applications in drug delivery: Albers and Muller (1995), Loftsson and Brewster (1996), Rajewski and Stella (1996), Irie and Uekama (1997), Thompson (1997), Medlicott et al. (1998), Ma et al. (2000); Zhao et al. (1999, 2002); Uchenna Agu et al. (2005). Information on SBEβCD (known for its product name Captisol) can also be found at Cydex Inc.'s website: http://www.cydexinc.com. Commercial product examples for HPβCD include Sporanox (Itraconazole, i.v.) and MitroExtra (Mitozytrex, i.v.); for SBEβCD (Captisol): Geodon (Ziprasidone mesylate, i.m.), Vfend (Voricanazole, i.v.), Abilify (Aripiprazole, i.m.), etc.

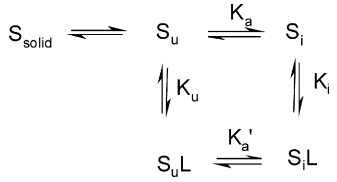
Cyclodextrins increase the drug solubility by forming a water-soluble drug-ligand (cyclodextrin) complex. The total solubility $[S_{tot}]$ for a one-to-one drug-ligand complex (A_L type) can be shown as follows:

$$[S_{tot}] = [S_0] + \frac{K[S_0]}{1 + K[S_0]}[L_{tot}]$$

where $[S_0]$ is the drug solubility in water, $[L_{tot}]$ is the total ligand concentration, and K is the drug-ligand complex stability constant.

In animal studies, both HPβCD and SBEβCD are broadly used in different routes for various animal species. For example, 20–40% (w/v) of these excipients are frequently used in a single-dose for either oral or i.v. route (see Table 4).

Cyclodextrins can be used in combination with pH adjustment for synergistic drug solubility enhancement. The following shows the scheme for the equilibrium established among a few major species.



$$[S_{tot}] = [S_u] + [S_i] + [S_uL] + [S_iL]$$

where $[S_u]$ is unionized drug, $[S_i]$ is ionized drug, $[S_uL]$ is unionized drug-ligand complex, and $[S_iL]$ is ionized drug-ligand complex; K_a is the drug ionization constant, and K_u and K_i are stability constants for unionized drug-ligand, and ionized drug-ligand complex, respectively. This modeling explains that the synergistic effect is generated due to the newly formed species, the ionized drug-ligand complex $[S_iL]$, which is absent in situations where pH adjustment ($[S_u] + [S_i]$) or cyclodextrins is used alone ($[S_u] + [S_uL]$). A variety of studies were published documenting the effective approach of this combined solubilization technique (cyclodextrin with pH control) in improving drug solubility (Liu et al., 1992; Tinwalla et al., 1993; Johnson et al., 1994; Okimoto et al., 1996; Li et al., 1998).

Loftsson et al. (1994) reported that addition of small percentage of hydrophilic polymers in cyclodextrin-based formulation can further enhance drug solubility. For example, with addition of 0.25% (w/v) polyvinylpyrrolidone (PVP), the solubility of a number of drug compounds were increased from 12 to 129% in a 10% (w/v) HPβCD vehicle (other related literature: Loftsson

et al., 1996; Loftsson and Fridriksdottir, 1998). The most recent example was by Cirri et al. (2006) who used a combination of cyclodextrins, pH and hydrophilic polymers to study Naproxen solubilization. It was found that addition of PVP allowed an increase of the drug-ligand (HPβCD) stability constant up to 60% as compared to corresponding drug-ligand binary system. We also discussed a case study using this approach (see Section 5.2).

Hydroxylic acids or bases can be used in drug-cyclodextrin solutions to enhance the drug solubility through formation of a 'multi-component complex' (Redenti et al., 2000; Redenti et al., 2001). A number of techniques were reported for preparing this 'multiple-component-complex' including precipitation, freeze-drying, or spray-drying. The resulting amorphous solids were found to dissolve rapidly and to generate a supersaturation that is stable for several days. Common hydroxylic acids used for this purpose are citric acid, lactic acid, malic acid, tartaric acid; hydroxylic bases may include tromethamine, diethanolamine, triethanolaime, etc.

3.4. Use of surfactants

Surfactant and micellar system can play one or multiple roles. It enhances drug solubility, improves the drug particle wetting and dissolution, reduces or eliminates drug precipitation, decreases drug degradation, modulates drug release, facilitates drug update, etc. Surfactants are used in many dosage forms: solutions, colloidal systems (emulsions, microemulsions, etc.), capsules/tablets, etc. They are widely seen in commercial oral and parenteral products, though often at a low concentration.

In his book *Surfactants and Polymers in Drug Delivery*, Malmsten (2002) extensively reviewed physical chemical aspects of surfactants, the self-assembly structures, and the examples in delivering challenging drug compounds. A simple equation illustrates the principle of surfactant induced-micellization and its impact on drug solubilization:

$$[S_{tot}] = [S_0] + \kappa \cdot [S_0] \cdot [S]$$

where $[S_{tot}]$ is the total solubility, $[S_0]$ is the drug aqueous solubility, $[S]$ is the surfactant concentration, and κ is the partition coefficient.

A major concern for conventional surfactants is that they cause systemic toxicity including histamine release and adverse cardiovascular effects. For example, Lorenze et al. (1977) reported significant histamine release in dogs as a result of using Cremophor EL. Attwood and Florence (1983) published toxicology data for many surfactants, a compilation of literature data prior to 1983. It is also noted that many conventional surfactants are known to interfere with *in vivo* biological process such as GI permeability enhancement, inhibition of efflux transporter P-glycoprotein (P-gp). Though these effects can be exploited in certain applications, their overall impact need to be closely monitored and be understood.

In early formulation development, surfactants are appropriate for low dose applications. Conventional surfactants include polysorbates (e.g.: Tween80, Tween20), polyoxyl castor oil (Cremophor EL, Cremophor RH40, RH60), etc. These surfactants can be used alone or with pH control. Combined use of pH with surfactants was reported to significantly increase drug solubility. Li et al. (1999a,b,c) discussed this approach using polysorbate 20 on drug Flavopiridol. Jain et al. (2004) discussed sodium lauryl sulfate with pH control on drug PG-30095. Li and Zhao (2003) proposed a new model to describe the solubilization phenomenon of drug Flurbiprofen by combined use of surfactants and pH adjustment. Unlike cosolvents system, drug formulated by surfactants are least likely to precipitate upon dilution *in vivo*.

Use of bile salt micelles/lethicin, or mixed micelles is another useful approach (see reviews by Wiedmann and Kamel, 2002). Common bile salts include taurocholate (TC), taurodeoxycholate (TDC), and deoxycholate (DC), all of which vary in the number of hydroxyl groups on the steroidal core structure. Common lipids include egg or soy phosphatidylcholine, soy phosphatidylethanomine, oleic acid, monoglycerides, etc.

Over the last two decades, there has been steady progress in developing new surfactants with improved solubilization, biocompatibility, and safety profiles. These are primarily from lipid-based or polymer-based functional excipients. Notable examples include Solutol HS-15 (macrogol-15-hydroxystearate), VitE-TPGS 1000 (*d*-α-tocopheryl polyethyl glycol 1000 succinate), Pluronic F68 (also known as Poloxamer 188, a block polymer of 81% poly-ethylenglycol and 19% of polypropylenglycol), Gelucire 44/14 (lauroyl macrogol-32 glycerides), etc. These surfactants are broadly reported as having high tolerability both in oral and in i.v. Many of these excipients were already introduced into commercial products. For example, Solutol HS-15 is used as a powerful solubilizer in injectable solution formulations up to 50% in Panitol (for Propanidid).

Another progress is to prepare ultra-purified brand for known conventional surfactants such as polysorbates and lecithin. For example, NOF Corporation developed a ultra-purified polysorbate 80 or Polysorbate 80 (HX)^{TM}. It was reported that this highly purified surfactant has minimum allergic reaction or histamine release as compared to several other polysorbate 80 products on the market (http://www.nof.co.jp).

3.5. Suspension, nanosuspension

3.5.1. Suspension

Suspension is broadly used in all animal studies. A conventional suspension may be readily prepared in an aqueous solution with small percentage of hydrophilic polymers, as well as small percentage of surfactants. The polymers are used for particle suspending and homogeneity while surfactants are for particle wetting and dispersing. Examples for polymers include methyl cellulose (MC), hydroxylethyl cellulose (HEC), or hydroxypropyl cellulose (HPC); examples for surfactants include polysorbate 80, polysorbate 20, Solutol HS 15, etc. For example, a suspension may contain 0.5% (w/v) HEC and 0.2% (w/v) polysorbate 80.

Physical stability is a major concern for suspension preparation and short-term storage. Issues such as change in particle morphology, particle aggregation, sedimentation will all have bearings on drug dissolution and bioavailability. In many situa-

tions, a milled or micronized suspension is preferred because it provides improved dissolution as well as batch-to-batch reproducibility. There are many techniques for preparing lab-scale milled or micronized suspensions such as wet-milling, high-pressure homogenization (microfluidization), etc. (see a case study in Section 5.1).

3.5.2. Nanosuspension

A new variant, nanosuspension shows significant improvement in enhancing drug dissolution and bioavailability. With particles in nano-range and narrow distribution, nanosuspension also shows significant improvement in its physical stability and batch-to-batch reproducibility. In principle, nanosuspension can be prepared via a variety of techniques: wet-milling, high-pressure homogenization, spray drying, solvent precipitation, supercritical fluid technology, etc. In recent years, there developed a number of technology platforms with improved technical controls: DissoCube (high-pressure homogenization, by SkyePharma), Nanopure (homogenization, by PharmaSol), Nanocrystal (wet-milling, by Elan), Nanoedge (homogenization and microprecipitation, by Baxter), etc. Nanocrystal is one of the most noticeable technology. At the core is the NanoMill process that utilizes high cross-linked polystyrene beads as grinding material (Liversidge et al., 1991), together with selection of surfactants and polymers in the formulation. Two oral products are in US market bearing Nanocrystal technology: Rapamune for Sirolimus, and Emend for Aprepitant.

As a dosage form, nanosuspension can be used in early formulations for challenging drug compounds via lab-scale NanoMill process (<50 ml). The formulation can be developed for various routes including oral and i.v. Among others, special attention needs to be paid to vehicle selection (choice of surfactants and polymers), particle size (in general, <600 nm for oral; <300–400 nm for i.v., though <200 nm is preferred), formulation physical stability, etc. In order to produce a high-quality nanosuspension, one also needs to develop a set of process parameters such as milling speed and grinding time.

3.6. Emulsion, microemulsion

3.6.1. Emulsion

Emulsion is a colloidal system that contains either oil-in-water (o/w) or water-in-oil (w/o) particles stabilized by surfactants in interfacial phases. For poorly water-soluble drug compounds, the o/w type has broader implications. Among earliest publications, Armstrong and James (1980) broadly reviewed drug release from lipid-based dosage forms. As a delivery system, emulsion is a mature technology. One of the commercial products is Propofol for Diprivan which is used in anesthetics or sedation (i.v. bolus and infusion). Emulsion is also broadly used in nutritional products including Intralipid, Nutralipid, Liposyn and Lipofundin. These products are developed and used primarily for nutritional substitute via i.v. injection. They have similar composition: soybean oil, lecithin (phospholipids), glycerin, etc., and buffered at approx. pH 7 ± 2.

For animal studies (especially via oral dosing), one can develop an emulsion formulation by evaluating drug solubility in common lipids/surfactants or available emulsion vehicles from literatures. One jump-start is to use the above nutritional products such as Intralipid or Lipofundin. One can first dissolve the drug in the emulsion product followed by vortexing and sonication. Alternatively, one can dissolve the drug in small amount of cosolvents (e.g.: ethanol), or polar lipids (e.g.: diacetylated monoglycerides), or a mixture of both, then mix it with emulsion product (Zhao, unpublished data).

3.6.2. Microemulsion

Microemulsion is a significantly improved version of emulsion with high promise in delivering BCS (biopharmaceutical classification system) Class II and IV compounds. A large volume of literature is available devoting to the principles and practice of this dosage form. Pouton (1985) evaluated the potential of self-emulsifying drug delivery systems. Humberstone and Charman (1997) discussed the beneficial effects of food or oils on bioavailability for lipophilic drug compounds. A few studies provide extensive coverage on the progress and opportunities on lipid-based formulations as a viable dosage form for bioavailability enhancement (Charman, 2000; Porter and Charman, 2001).

A common version for oral microemulsion is the self-(micro)emulsifying drug delivery system or SEDDS, SMEDDS. This technology has been introduced into the market product Neoral (by Novartis). A microemulsion-generating oral formulation, it significantly improves drug Cyclosporine A *in vivo* performance: enhanced bioavailability (50% in humans), low variability and little food effects. This contrasts to the previous emulsion-generating oral product Sandimmune, which has a BA of 30% in humans, high variability, and food effects (http://www.pharma.us.novartis.com/product; Pollard et al., 2003; Humbert, 1997).

In general, microemulsion offers many advantages as compared to emulsions: small particles (nano-range, often <150 nm), thermodynamic stability, and potential to improve bioavailability. It is worth mentioning that, despite all the benefits, microemulsion as well as SMEDDS is still a relatively novel delivery system. There have been many unknowns and challenges: the *in vivo* mechanism for bioavailability enhancement, formulation development and optimization process/criteria, large-scale manufacturing, physical stability, etc.

In early formulations, microemulsion (including SMEDDS) can play an important role: there has been a large array of available excipients including those GRAS (generally regarded-as-safe) and recently developed functional lipids and surfactants. In general, selection of oil phase may focus on digestible ones such as free fatty acids, mono-, di- or tri-glycerides and derivatives, mono-, di-propylene glycol fatty acid esters, etc.; selection of surfactants may include polyethoxlated fatty acids (e.g.: Cremophor EL), polyglycerol fatty acid esters, other fatty acid derivatives (e.g: polysorbates, Span 80), etc.

The roadmap to developing microemulsion/SMEDDS involves a variety of physical chemical characterization: particle morphology, particle size and distribution, dispersion/dissolution, physical stability over stress conditions, etc. There has been a large volume of literature discussing formu-

lation development and the subsequent in animal PK studies. In a recent study, Shen and Zhong (2006) discussed SMEDDS formulation for atorvastatin, a low solubility compound with low oral BA. The SMEDDS in capsules consists Labrafil, PG, and Cremophor RH40. The PK study in 6 beagle dogs after oral dosing (mg/kg) found that the drug BA was significantly increased compared with to that of conventional tablet. Recently Gao and Morozowich (2006) investigated supersaturatable self-emulsifying drug delivery system (s-SEDDS) formulations for improving the oral absorption of poorly soluble drugs. In the case study, we will also discuss a SMEDDS formulation development for a preclinical candidate that involves excipient selection, pseudo-phase diagram construction, and formulation characterization (see Section 5.4).

3.7. Liposome

Liposome is made up of one or more concentrically arranged phospholipid bilayer structures: the hydrophilic heads of the phospholipid molecules are towards aqueous phase (internal or outside), while lipophilic tails are towards each other. Due to the unique structure, liposome can be used to deliver diverse drug compounds: hydrophilic ones are in internal aqueous core, lipophilic ones in lipidic bilayer, and amphiphilic ones adsorbed onto the double lipidic membrane. Liposome can be classified into various groups of vesicles depending on structures/particle sizes as the followings being three major ones:

(a) Small unilamellar vesicles (SUV): 20–100 nm.
(b) Large unilamellar vesicles (LUV): >100 nm.
(c) Multilamellar vesicles (MLV): >500 nm.

Due to the excipients used, liposome is generally considered biocompatible and tolerable. Literatures on liposome as a drug dosage form are prevalent (see reviews/books: Langner and Kral, 1999; Betageri et al., 1993; Lasic, 1993; Betageri and Parsons, 1992). Liposome has been successfully brought into commercial products both in US and in Europe. Examples include DaunoXome for Daunorubicin Citrate (NeXstar), Doxil for Doxorubicin (Sequus), Ambisome for Amphotericin B (NeXstar), etc.

As it involves complex preparation and physical stability issues, liposome is one of the last approaches in early formulation development. The formulation can be prepared in a lab-scale for animal dosing, mostly in i.v. route or routes bypassing GI tract such as i.a. (intra-articular). Currently there are two common preparation methods:

Thin-film hydration

This is the most common preparation method: it starts with dissolving drug and lipids (e.g.: phospholipids and other lipids/cholesterol) in organic solvents. Common solvents include chloroform, ethanol, methanol, or mixture of chloroform and methanol which provides good solubility to phospholipids. This is followed by solvent removal (e.g.: rotary evaporation), which leads to formation of thin-film containing drug and lipids on the wall of the flask. The thin-film is then hydrated with aqueous buffers: gentle shaking of the flask results in homogeneous milky-like liposome suspensions. It is of note that the liposomes prepared at this stage are often MLV. Further particle size reduction is necessary to meet i.v.-acceptable standards. Methods for sizing down the particles include sonication, homogenization, microfluidization, membrane extrusion, etc. Zhao et al. (2000) reported that, for small preparation (<10–20 ml), membrane extrusion with final liposome particle size in 100–200 nm and short-term physical stability (2 weeks, 37 °C) were achievable. Ran and Yalkowsky (2003) reported that thin-film hydration preparation using halothane shows improvement for the liposome containing AMPB, an anticancer drug, both in particle size and short-term physical stability.

Solvent injection

This is a less-frequently used method, as compared to thin-film hydration. The method starts with dissolving the drug/lipid excipients in low-boiling solvent(s). The organic solution is then injected (drop-by-drop) into an aqueous phase (probably containing buffer) while maintaining vortexing the aqueous solution (in order to remove the solvent as it drops).

3.8. Solid dispersion

Solid dispersion is the dispersion of the drug compound in an inert carrier at solid state. For formulations targeting dissolution and bioavailability enhancement, solid dispersion often takes the form of 'solid solution', where the drug is molecularly dispersed in a hydrophilic polymer via preparations such as solvent evaporation or hot-melt extrusion. Both methods need further processing for commercial product development. Among early investigators are Sekiguchi and Obi (1961), Simonelli et al. (1969), Chiou and Riegelman (1969), etc. For example, Chiou and Riegelman (1969) prepared the solid dispersion by dissolving drug Griseofulvin in PEG6000 via solvent evaporation method. Serajuddin et al. (1988) prepared solid dispersions for REV-5901, a poorly water-soluble drug, in different PEGs and in Gelucire® 44/14 filled in hard-gelatin capsule. A recent example was reported by Matsunaga et al. (2006) who used solid dispersion formulations to improve drug KRN633 dissolution and bioavailability: the bioavailability was increased 7.5-fold in rats relative to the pure crystalline drug form. The technology has also been introduced into commercial products. Examples include Gris-PEG for Griseofulvin (Novartis), Cesamet for Nabilone (Lilly), Kaletra for Lopinavir/Ritonavir (Abbott), etc., all of which are oral solid dosage forms (tablets).

To prepare an acceptable formulation, it is important to ensure that the drug remains amorphous state over stress conditions. It is therefore necessary to address relevant choices and issues: polymer, preparation method, drug payload, incorporation of other excipients such as surfactants or disintegrants, etc.

Choice of the polymer: a variety of polymers or even surfactants can be used for solid dispersion formulations: PVP (polyvinylpyrrolidone), HPMC (hydroxypropylmethylcellulose), HPMC phthalate, HPMC acetate succinate, PEG4000, Pluronic F68, PEG3350, Gelucire 44/14, etc. Many polymers have various categories such as PVP and HPMC due to variation in molecular weight. For example, PVP K-12, K-30, K-90, etc. With increasing MW, the viscosity of the polymer in solu-

tion also increases which can be plus or minus depending on applications.

Choice of preparation: a common method for lab-scale preparation (<1–10 g) is to use solvent evaporation: it starts by dissolving the drug and the hydrophilic polymer (with or without other excipients) in a low boiling organic solvent followed by evaporation of the solvents and complete drying process. It is important to evaluate the solubility of the drug and the polymer in these organic solvents, and select the most appropriate one for preparation. Common solvents include dicholoramethane, methanol, ethanol, isopropanol, acetone, ethyl acetate, or the combination of these solvents. Ethanol may not be an ideal solvent for poorly water-soluble drug compounds, but proves to be a good solvent for many hydrophilic polymers (Zhao, unpublished data).

For early formulation development, solid dispersion may not be the first choice due to the work involved in the formulation selection and preparation discussed above. However, it can be an effective approach to seek greater *in vivo* exposure when one or more of the followings exist: (a) difficult drug compound due to poor solubility/dissolution; (b) high dose required; (c) other formulation approaches are not adequate.

4. Developing early formulations

4.1. Starting point

To develop an early formulation, one needs to garner all relevant information: study purpose, dose, route, formulation requirement, animal species, drug compound status, etc. In addition, one needs to have a good knowledge on sample purity, amount available, development timeline, etc.

Basic physical chemical properties are the keys to understand the compound at hand and potential formulation options. These properties include chemical structure, pK_a, log P/log D, solubility, stability, melting point, crystal/amorphous, particle size/distribution, etc. Knowledge on biological aspects is also useful: gastro-intestinal (GI) permeability represented by Caco-2 or by PAMPA (parallel artificial membrane permeability assay), metabolic stability, active transport system or P-glycoprotein-drug interaction (P-gp), etc.

4.2. In silico assessment

In silico assessment is useful prior to actual experimentation, in particular when sample amount and timeline are restricting factors. It provides information on a range of physical chemical properties. Table 3 provides a number of common *in silico* software used in drug discovery and development. The soundness of software largely depends on the database upon which the mathematical modeling is built. For example, the $C\log P$ software, which was initially developed by Hansch and Leo (1979), now part of Bio-Loom program, has a prediction scheme based on a database containing 60,000 measured log P/log D and 14,000 pK_a data (http://www.biobyte.com).

There is also a large body of useful information in the public domain that can directly or indirectly benefit early formulation development: Lipinsky's 'Rule of Five' (Lipinski et al., 1997) was originally proposed to raise awareness about structural features and less drug-like properties. He stated that compounds that fall in the following category are likely to have oral absorption/permeability issues:

(a) Molecular weight ≥500
(b) Log P ≥5
(c) H-bond donor ≥5
(d) H-bond acceptors ≥10

The general solubility equation (GSE), proposed by Yalkowsky and co-workers (Yalkowsky and Valvani, 1980; Yalkowsky, 1999; Jain and Yalkowsky, 2001), can predict aqueous solubility S with knowledge of melting point T_m and octanol–water partition coefficient log P as shown below:

$$\log[S] = -0.01(T_m - 25) - \log P + 0.5$$

In fact, both melting T_m and partition coefficient log P ($P = K_{octonol/water}$) can be calculated based on drug chemical structures. For example, for melting point calculation, there are many schemes or prediction models available including group contribution method (see references: Dearden and Rahman, 1988; Krzyzaniak et al., 1995; Zhao and Yalkowsky, 1999).

4.3. Solubility assessment

Solubility enhancement is the single most important driving force in early formulation selection and optimization. Table 4 provides a recommended excipient(s) use range for oral and i.v., and primarily serves for solution formulation preparation. This is primarily for solution formulations. The list can also be seen as an example of drug solubility assessment. As a general rule, if solubility is <1% (w/v) or 10 mg/ml in the aqueous or buffers, it is necessary to further evaluate solubility in pharmaceutical excipients and vehicles.

Table 3
Examples of *in silico* softwares

Software	Main features	Developers
ACD LogD Suite	Solubility/pH, log D/pH, pK_a, etc.	Advanced Chemical Development Inc.
QikProp	Solubility, log P, blood/brain partition coefficient (log BB), Lipinsky's rule of five, polar surface area (PSA), molecular volume, Caco-2 permeability (P_{Caco}), metabolism	Schroedinger Inc.
Pipeline Pilot	log D/pH, solubility, polar surface area (PSA), pK_a	SciTegic Inc., now part of Accelrys Inc.
Bio-Loom	log P, log D/pH, pK_a	BioByte Inc.

Table 4
Recommend excipient(s) use range in early formulations for oral and i.v.

	Solvent or excipient with full name[a]	Dosing route/common usage range[b]
Aqueous	Water	oral, i.v.
	0.9% NaCl	i.v.
	D5W – 5% dextrose in water	i.v.
	Buffered solutions pH: 2–8	oral, i.v.
Cosolvent	NMP – *N*-methylpyrrolidon	10–20% (oral, i.v.)
	DMSO – dimethyl sulfoxide)	10–20% (oral or i.v.)
	Ethanol	10% (oral, i.v.)
	DMA – *N*,*N*-dimethylacetamide	10–30% (i.v.)
	PG – propylene glycol	30–60% (oral, i.v.)
	PEG400 – polyethylene glycol 400	40–100% (oral, i.v.)
	Transcutol – diethylene glycol monoethyl ether	30% (oral)
Cyclodextrin	HPβCD – hydroxyl-β-cyclodextrin	20–40% (oral, i.v.)
	SBEβCD – sulfobutylether-β-cyclodextrin	20–40% (oral, i.v.)
Surfactant	Posorbate 80 (Tween 80) – polyoxyethylene-sorbitanmonooleate 80	5–10% (oral, i.v.)
	Cremophor EL – polyoxyl-35 castor oil	5–10% (oral, i.v.)
	Cremophor RH40 – polyoxyl 40 hydrogenated castor oil	5–10% (oral, i.v.)
	Sodium cholate	10–20% (oral, i.v.)
	Pluronic F68 – or Poloxamer 188: 81% polyethylene glycol and 19% of polypropylene glycol	20–50% (oral, i.v.)
	Solutol HS-15 – macrogol-15-hydroxystearate	20–50% (oral, i.v.)
	VitE-TPGS 1000 – *d*-α-tocopheryl polyethyl glycol 1000 succinate	20–50% (oral)
	Gelucire 44/14 – lauroyl macrogol-32 glycerides	20–50% (oral)
	Labrasol – caprylocaproyl macrogol-8-glycerides	40–60% (oral); 20–40% (i.v.)
	Lecithin – phosphatidylcholin	20–50% (oral, i.v.)
Lipid	Soybean oil	50–100% (oral)
	Miglyol 812 – mid-chain triglyceride of caprylic/caprolic acid	60–100% (oral); 20–40% (i.v.)
	Labrafil 1944CS – polyxoyethyllated oleic glycerides	30–60% (oral, i.v.)
	Capmul MCM – medium chain mono- and diglycerides	30–60% (oral, i.v.)

[a] Most of the cosolvents, surfactants or cyclodextrins can be used in combination with pH adjustment in weak base/acid drugs for synergistic solubility enhancement.
[b] The concentration range is based on mouse or rat model; administration volume is approx. 10 ml/kg for a single dosing.

A common technique for solubility measurement was developed by Higuchi and Connors (1965), and has been broadly used ever since due to its simplicity. An experimental procedure is listed as follows:

(a) To the vial containing test solutions/vehicles, add sufficient amount of drug solids.
(b) Equilibrate via shaking/rotating the sample vial at a given temperature: ambient temperature or 25 or 37 °C.
(c) Filter the solution when the drug concentration in the vial reaches saturation, i.e. solids are still present.
(d) Filter the solution; assay the filtrate by HPLC (dilute the filtrate if needed).

In absence of definitive time for drug saturation, it is common to conduct the solubility assessment at ambient temperature over 24 or 48 h.

A practical concern in solubility measurement is that one needs to handle the sample properly without producing supersaturation, a phenomenon that often leads to an over-estimation of the solubility value. One exercise of caution is not to overheat or sonicate the samples (especially those difficult to wet in solution) as an initial effort to get it into solutions. Discrete approaches may include vortexing (not vehemently), addition of small amount of surfactants (<1% polysorbate 80, which has negligible effect in solubilizing, but substantial in wetting), small amount of ethanol coupled with physical mixing prior to adding the mixture into solutions, etc. Supersaturation, once formed, proves to be sustainable in some situations (Zhao, unpublished data).

In the following discussion (Section 4.6), we will discuss another measurement for solubility, or kinetic solubility. This is a less accurate but more practical approach in everyday early formulation development work.

4.4. Strategies for formulation development

Early formulation development is largely dealt with individually. There have been some reports addressing the need for a more systematic approach with regard to solubility enhancement. Bittner and Mountfield (2002) discussed a flowchart composed of aqueous, buffered pH, cosolvents, cyclodextrins, nano- and micro-suspensions, mixed micelles, emulsions, etc. Lee et al. (2003) investigated many Pfizer's discovery drug compounds and found that out of >300, 85% were formulated using pH adjustment, cosolvents or the combination of both. They thus proposed a decision tree for working with these compounds primarily using pH adjustment and cosolvent approaches. In a separate discussion on solubilizing excipients in commercial oral and parenteral products, Strickley (2004) described a

flowchart suggesting the order of solubilization approaches for injectable and oral liquid formulations. Though it is not specifically for animal formulations, it nevertheless provides useful thinking process.

In this study, we propose two more general schemes: one for oral (Fig. 1) and the other for i.v. (Fig. 2). These schemes are intended to strategize our efforts in working with early formulations with solubility enhancement techniques at its core. In both schemes, it is emphasized that drug physical chemical properties as well as other relevant information be acquired prior to development work. *In silico* assessment on major physical chemical properties (e.g.: log P, pK_a) is recommended in the absence of experimental data.

A few key points to Fig. 1 are listed as follows:

(a) For oral solution formulation, it starts with aqueous, buffers; if there is no good solubility, move to other solution approaches: cosolvents, cyclodextrins, micelles. Pay special attention to the combined use of pH with these excipients.
(b) If solution vehicles are not appropriate (or no adequate solubility), one needs to move to novel formulations such as nanosuspensions, solid dispersions, emulsions, etc. The choice of these dosage forms largely depends on the drug compound as well as the study/project need.
(c) For suspension (right side of Fig. 1), there are some options: conventional suspension, micronized suspension. Due to its reduced particle size and increased homogeneity micronized suspension provides potential for improved dissolution as well as control on batch reproducibility.

Also, a few key points to Fig. 2 are listed as follows:

(a) For i.v. solutions, there is a similar development sequence: aqueous, buffers, followed by use of cosolvents, cyclodex-

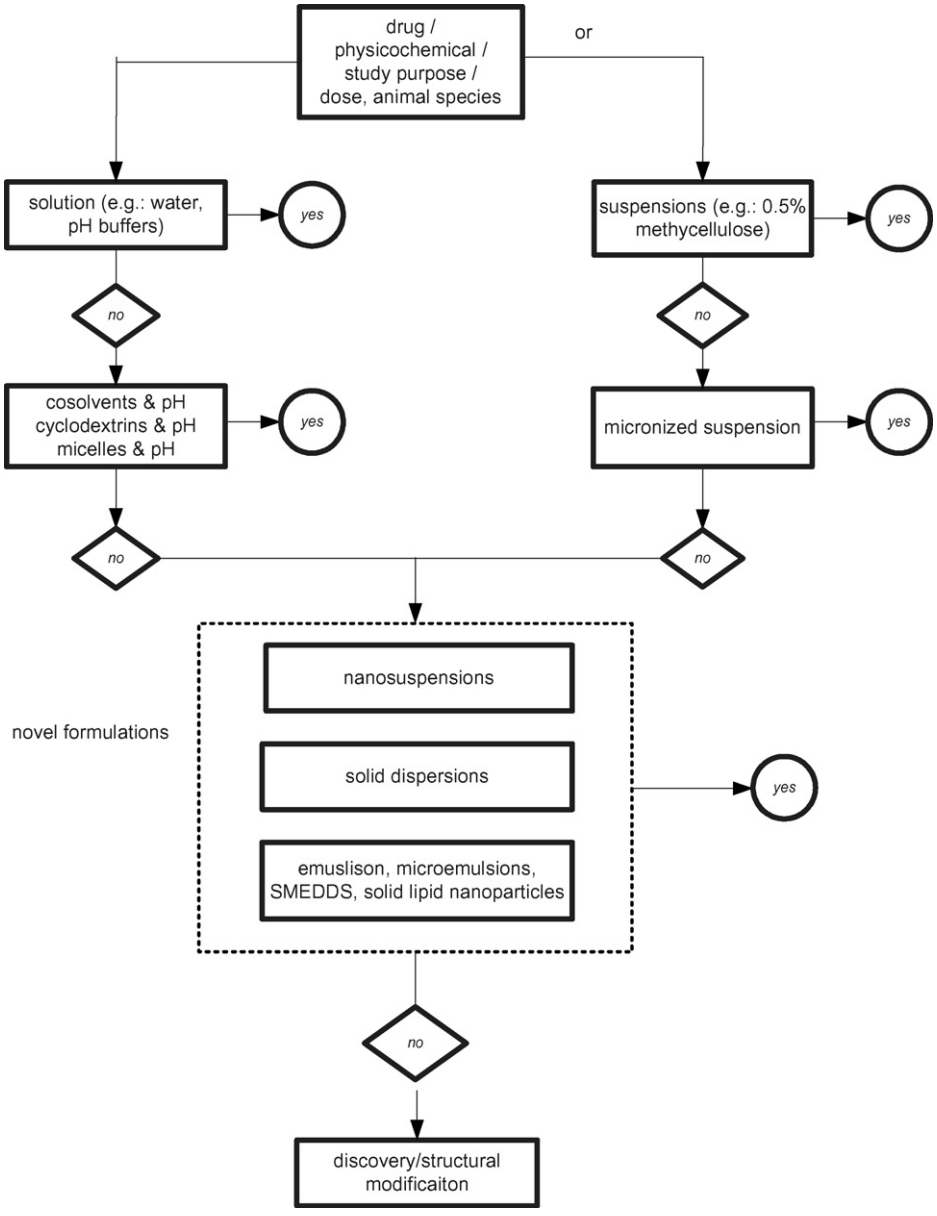


Fig. 1. Developing early formulations for oral route.

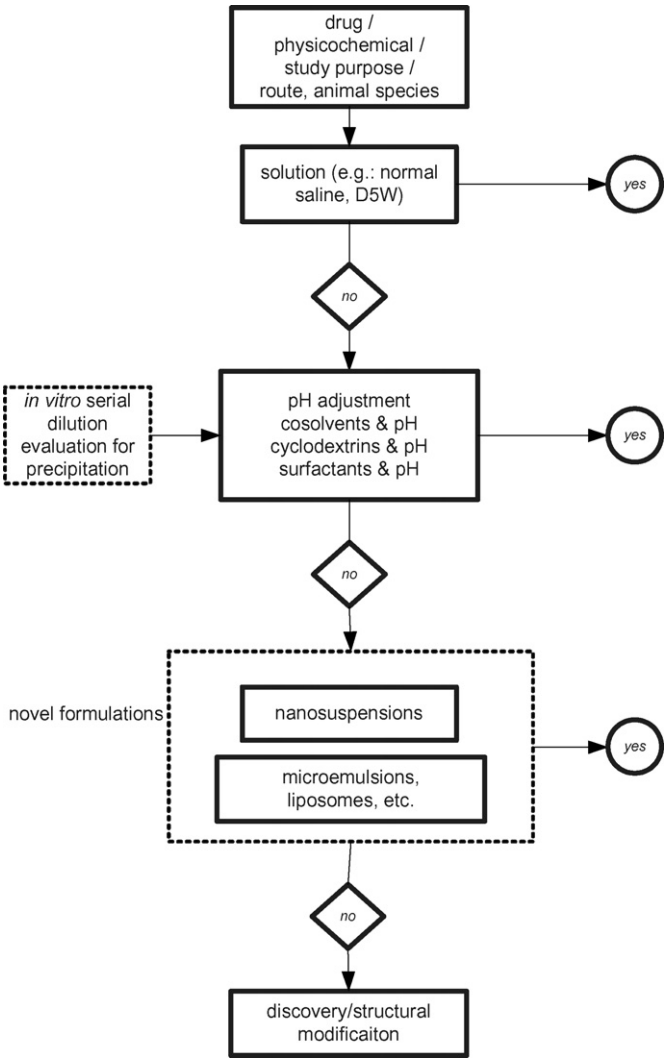


Fig. 2. Developing early formulations for i.v. route.

trins and micelles, and the combined use of pH with these excipients. The combined use of pH with cosolvents or with cyclodextrins is particularly effective.

(b) For i.v. formulations, special attention needs to be paid to drug precipitation upon injection. It is advised to evaluate the formulation *in vitro* precipitation by using serial dilution method (see discussion below, Section 4.5), and to use the technique as a guiding tool for formulation optimization (minimizing or eliminating precipitation). For an acceptable i.v. solution formulation, it is important to ensure that the drug remain solubilized before and upon injection.

(c) For animal i.v. application, solution formulation is the primary dosage form. In situations where the solution formulation is not able to address the need, one may begin to consider nanosuspensions, microemulsions, etc.

In both Figs. 1 and 2, novel formulations are generally reserved for challenging drug compounds. By 'challenging', we mean that these compounds were not able to be formulated by aqueous, cosolvents, cyclodextrins, and simple micelles, or any of these combinations. If the pursuit of greater *in vivo* exposure is justified, one should then carefully evaluate pros and cons of each novel formulation relating to the particular drug compound and formulation needs. The development work of any novel formulation, be it microemulsion or solid dispersion or nanosuspension, generally requires more time and commitment than conventional ones, and needs to be well-planned in the overall timeline projecting.

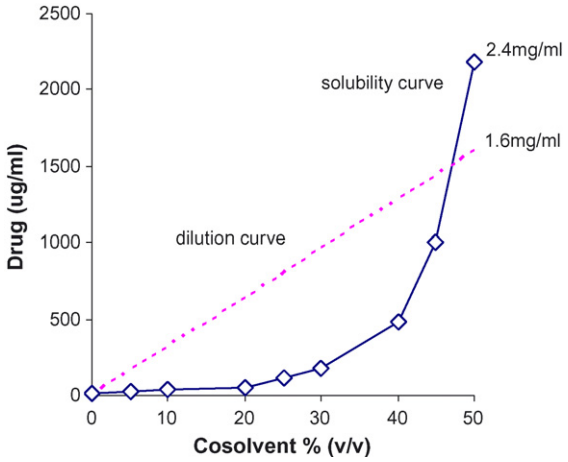


Fig. 3. Illustration of drug precipitation: solubility curve vs. dilution curve.

4.5. Precipitation potential evaluation

Precipitation upon dosing (mostly injection) remains a significant challenge for solution formulations, especially when they contain cosolvent(s). As discussed in Section 3.2, the supersaturation formed upon formulation via diluting with biological fluids (blood, gastric fluid, etc.) is the driving force responsible for precipitation.

Fig. 3 gives an illustration: the drug solubility is 2.4 mg/ml in a 50% cosolvent system, while the drug concentration in the formulation is 1.6 mg/ml. When the formulation is diluted, the concentrations of both the drug and the cosolvent decrease linearly. What well-within-the-solubility-range at a higher cosolvent concentration suddenly becomes well-over-the-solubility-range at a lower cosolvent concentration, due to the fact that cosolvent increases the drug solubility on a semi-logarithmic scale (also see discussion in Section 3.2). There are two curves in Fig. 3: one is drug solubility curve, and the other is drug concentration curve based on dilution (or dilution curve). When the drug concentration is above solubility curve, this means that the drug is in a supersaturated state (or metastable state), and is prone to precipitation. Similar phenomenon can be observed in pH controlled formulations, especially those at low or high end with low buffer capacity (Simamora et al., 1995, 1996).

There are a number of *in vitro* methods available to assess drug precipitation in formulations. Irwin and Iqbal (1992) reported a dynamic evaluation method for bropirimine injections. Yalkowsky and co-workers (Ward and Yalkowsky, 1993; Yalkowsky et al., 1998) described a simple *in vitro* serial dilution method, which mimics an *in vivo* physiological dilution process. The method uses isotonic Sorensen's phosphate buffer (ISPB)

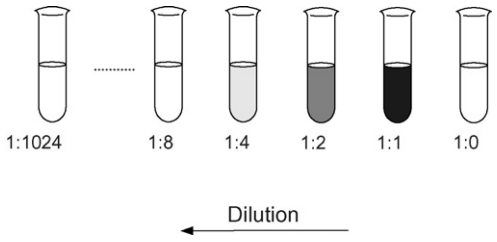


Fig. 4. Illustration of *in vitro* serial dilution for evaluating precipitation potential.

(pH 7.4, Na_2HPO_4–NaH_2PO_4 buffer) with a concentration as 0.067 M. It has a buffer capacity of 0.036, a value that is consistent with the buffer capacity of fresh whole blood (0.032–0.039) (Surakitbanharn et al., 1994).

Based on published literature by Yalkowsky's group, we propose an experimental procedure for *in vitro* serial dilution method as below:

(a) To 10 test tubes, add 0.5 ml ISPB each.
(b) To tube 1, add 0.5 ml formulation; shake gently to mix well.
(c) Transfer 0.5ml from tube 1 into tube 2, shake gently to mix well.
(d) Repeat the above step all the way to tube 10, at which point the original formulation in tube 1 is diluted 1024 folds (see Fig. 4, modified from Li et al., 1998).
(e) Observe for cloudiness/precipitation in all tubes.

The rate and extent of precipitation can vary, both of which are important indicators in predicting potential drug precipitation *in vivo* upon injection. If the precipitation is not to occur within a short time period (e.g.: a few minutes), the formulation is considered less likely to precipitate *in vivo* due to rapid physiological dilution by blood flow.

To make good use of this method, we propose the following assessment criteria for formulation precipitation potential:

(a) The drug is unlikely to precipitate, if no cloudiness/precipitation is observed in all 10 tubes in 3–5 min.
(b) The drug is less likely to precipitate, if slight cloudiness/precipitation is observed in one or more tubes in 3–5 min.
(c) The drug is likely to precipitate, if cloudiness or precipitation is observed in one or more tubes in less than 1 min. It is advised to further optimize the formulation.

In the case study (Section 5.3), we discussed an example of preparing cosolvent-based formulation for i.v. injection. In principle, one can optimize the formulation by incorporating small amount of surfactants or even hydrophilic polymers to minimize or eliminate potential precipitation.

The same *in vitro* serial dilution principle can be applied in oral solution formulation development. Here, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) are used as diluting medium in place of isotonic Sorenson's phosphate buffer (ISPB). Both SGF and SIF are listed in USP 30/NF 25. The preparation procedures are as follows: for 100ml SGF: use 0.2g NaCl in distilled water, adjusted the pH to 1.2 with hydrochloride acid (pepsin not added); for 100 ml SIF, add 0.68 g of potassium dihydrogen phosphate (KH_2PO_4) in distilled water, adjust the pH to 7.5 with sodium hydroxide (pancreatin not added).

4.6. Kinetic solubility measurement

As discussed, the sample amount available and the timeline can be determining factors in early formulation development. An alternative to equilibrium solubility, kinetic solubility can provide practically useful information. The measurement is rapid (e.g.: a few hours), and involves no use of HPLC or other instrumentation.

Listed below is an example procedure for measuring kinetic solubility in a cosolvent:

(a) Weigh the drug compound approx. 1 mg.
(b) Add 0.05 ml cosolvent, sonicate in water-bath at 25–30 °C for 1–3 min.
(c) If particle are not fully dissolved, add 0.05ml more cosolvent, followed by sonication at the same condition.
(d) Continue the above cycle until all drug particles are fully dissolved. For this particular measurement, there went three cycles before the drug was fully solubilized.
(e) Calculate kinetic solubility: $1\,mg/(3 \times 0.05\,ml) = 6.67\,mg/ml$.

Kinetic solubility is not robust as equilibrium solubility; frequently it yields falsely high solubility due to supersaturation. Yet it is practically useful, and provides much-needed solubility information in a variety of excipients and vehicles with small amount of sample, and in a timely fashion. Kinetic solubility can be broadly used in developing formulations containing cosolvent(s), cyclodextrin, lipids, and polymers (see Section 5.3).

4.7. Formulation stability: causes and approaches

Chemical instability is a frequent occurrence in working with early formulations. Sources to instability/degradation vary: solution pH, hydrolysis, temperature, light, oxidation, etc. When possible, it is important to investigate degradation mechanism, and to develop appropriate strategy. A good reference, Connors et al. (1986) investigated extensively on chemical stability mechanism with a large array of drug compounds.

Degradation at extreme pH

This is often observed for many drug compounds. If oral route is required, one may consider a buffered formulation at neutral or alkaline pH. One may also administer the drug via intraduodenal (i.d.) route, which bypasses the stomach and directly into the duodenal so as to avoid stomach's acidic environment. Another approach is to prepare a solid dispersion by incorporating enteric polymers. For example, a simple solvent evaporation method can be used for the preparation using hydrophilic polymers such as Eudragit L100, hydroxypropyl methylcellulose phthalate (HPMCP), etc.

Hydrolysis

Hydrolysis is another commonly observed phenomenon for chemical instability. The first-line approach is to use buffered

solutions at a pH where the drug stays stable. Other formulation approaches may also be considered with careful evaluation of the drug physical chemical properties: lipid-based formulations, nanoparticles, prodrug preparation, cyclodextrins, or even cosolvents-based formulations.

Formulation stability evaluation

In general, short-term stability is appropriate for early formulations. For a single-use solution formulation (oral or i.v.), chemical stability at ambient temperature in 24–48 h is sufficient; for suspensions or lipid-based formulations, both chemical stability and physical stability need to be evaluated. Physical stability includes visual observation, microscopic observation, particle size and distribution. For multiple or chronic-dosing, a more extensive stability evaluation is often needed in order to justify respective shelf-life during the animal dosing. A solution formulation with acceptable stability profile should be free from turbidity, precipitation, discoloring, etc. For suspensions or lipid-based formulations, it should be free from phase-separation, caking, lumping, de-coloring, etc. If settled (in the case of suspension), it should be easily suspended upon gentle shaking.

Recommended exercise of caution

A general exercise of caution is recommended in early formulation preparation:

(a) Prepare the formulation fresh prior to animal dosing whenever possible.
(b) Away from light: for short storage, keep the sample in dark, aluminum wrap, or in amber vial.
(c) Away from high or extremely low temperature: a cool/cold temperature at 5–10 °C is recommended for most formulation samples especially those lipid-based.
(d) Away from oxidation: if necessary, cover the formulation (e.g.: liposomes, emulsions) with nitrogen or argon. Certain lipids (e.g.: phospholipids) are particularly sensitive to oxidation.

4.8. Maximum tolerable dose for excipient(s)

The question of using maximum tolerable dose of excipient(s) without causing adverse effects to animals goes to the core of early formulation development. There appears to be no clear-cut answer, especially when the formulation is for chronic application (in toxicology study). In previous discussion, we presented a recommended use range for many excipients in solution formulation preparation (see Table 4), which is practically useful.

It is important to understand published data on excipients' tolerability and toxicology: acute toxicity, organ toxicity, GI side-effects, local tolerability (e.g.: s.c., i.v.). Many published LD_{50} data for various routes (e.g.: oral, i.v., s.c., i.p.) and for various animal species are available in literatures and in excipient handbook (e.g.: Handbook of Pharmaceutical Excipients, 4th ed., by Rowe, Sheskey and Weller, 2003). The LD_{50} is the lethal dose for 50% of those exposed to a toxic agent, and is frequently used as a general indicator of a drug compound's acute toxicity.

Commercial products can be a good resource for excipient(s) usage. Resources include: USP 30/NF 25 (2006), PDR (Physicians' Desk Reference) 57th ed. (2003), excipient handbook (see above), FDA's websites such as inactive ingredient guide (IIG). The IIG provides inactive ingredients in drug products, approved or conditionally approved, in US market. The same guide also provides route of administration and dosage form.

There are a number of useful reviews on excipients usage in commercial products. For example, Wang and Kowal (1980) reviewed excipients and pHs used in US parenteral products; Sweetana and Akers (1996) reviewed solubility principles and parenteral formulation applications; Strickley (2004) reviewed extensively on solubilizing excipients used in oral and injectable solution products which include cosolvents, non-ionic surfactants, cyclodextrins, phospholipids, water-insoluble lipids, etc. Table 5 provides a partial list compiled by Strickley's review paper on examples of cosolvents and surfactants in commercial injection products.

5. Case study

5.1. Case 1: suspension micronized by wet milling

The study was to evaluate and rank activity/efficacy for three discovery leads in guinea pigs via intra-tracheal (i.t.) route. The compounds are all weak bases (pK_a 3.6–4.1) with poor aqueous solubility as 0.1–0.5 μg/ml. The formulations were required to provide drug concentration as 2.5 mg/ml for each. Solubility assessment showed that it was impossible to prepare solution formulations for these compounds with normal pH range (pH 2–9). It was decided to use micronized suspension for each compound.

A common suspension vehicle was used: 0.5% (w/v) methylcellulose, 0.2% (w/v) Tween 80. A lab-scale wet mill (model: Retsch MM301) was utilized to prepare small volumes (6 ml) for each formulation. The grinding beads were polysterene in nature. The suspension was found homogeneous after milling via both visual and microscopic observation. Particle analysis (SympaTec particle analyzer) found that D_{90} was 10–16 μm for the particles in suspension. It was found that the suspension was stable after 3 days at ambient temperature with negligible chemical degradation (<1%) and essentially no change in particle size for all three compounds.

5.2. Case 2: use of pH adjustment and polymer addition in cyclodextrin-based formulations

The compound is a preclinical lead. The purpose of this study was to develop a high concentration solution-based formulation for dog PK profiling. The formulation was expected to be used for both oral and i.v. The compound is a weak zwitterion. The solubility: 12.2 μg/ml (pH 2.2), 1.2 μg/ml (pH 6.8), and 2.5 μg/ml (pH 9). The use of HPβCD was identified as an effective approach.

With combined use of cyclodextrin and pH control, the compound obtained substantial solubility enhancement: 4.5 mg/ml in 30% (w/v) HPβCD dissolved in 0.1M citrate. The final

Table 5
Examples of cosolvents and surfactants in commercial injection products

Excipient	% in marketed product	% administered	Route of administration	Product example
Cosolvent				
Ethanol	5–80	≤6	s.c.	Dihydroergotamine
		≤10	i.m.	Phenytoin
		≤10	i.v. (infusion)	Paclitaxel
		≤20	i.v. (bolus)	Paricalcitol
PEG300	≤60	≤50	i.m., i.v. (bolus)	Methocarbamil
PEG400	18–67	≤18	i.m.	Lorazepam
		≤9	i.v. (bolus)	Lorazepam
PG	10–80	≤80	i.m.	Lorazepam
		≤68	i.v. (bolus)	Phenobarbital
		≤6	i.v. (infusion)	Medroxyprogesterone
Glycerin	15–32	≤15	i.m., s.c., i.v.	Dihydroergotamine
		≤2.5	i.v. (infusion)	Idarubicin
DMA	6	≤3	i.v. (infusion)	Teniposide
Surfactant				
Cremophor EL	11–65	≤10	i.v. (infusion)	Paclitaxel
Cremophor RH60	20	≤0.08	i.v. (infusion)	Tacrolimus
Polysorbate 80 (Tween 80)	0.075–100	≤4	i.m.	Chlordiazepoxide
		12	i.m.	Vitamin A
		≤0.4	i.v. bolus	Amiodarone
		≤2	i.v. (infusion)	Docetaxel

From Strickley (2004).

solution pH was 3.2. Further study revealed that addition of small percentage of hydrophilic polymers such as PVP (polyvinylpyrrolidone), or Na-CMC (sodium carboxyl methylcellulose) variably increased drug solubility to additional 10–20%. The final formulations were as follows: (a) for oral formulation: 5.3 mg/ml drug concentration; 25% (w/v) HPβCD + 0.2% PVP (w/v) in 0.1 M citrate; the final pH 3.2. For i.v. formulation: 3.6 mg/ml drug concentration; 25% (w/v) HPβCD in 0.1 M citrate; the final pH 3.0. Both formulations were stable, physically and chemically, over 48 h at ambient temperature in darkness.

5.3. Case 3: use of surfactant in cosolvent(s) formulations to prevent precipitation

The compound is a preclinical lead. The study was to develop a solution formulation for toxicological evaluation via i.v. route to rats. The formulation was requested to provide a drug concentration as 2.0 mg/ml or higher. The compound is a weak base (pK_a 3.9), and has an aqueous solubility as 0.3 μg/ml. The pH adjustment at physiological pH (pH 2–9) did not produce sufficient solubility (e.g.: 11.5 μg/ml at pH 2.0). Cyclodextrin approach was not suitable due to structural incompatibility (e.g.: 0.1 mg/ml in 30% HPβCD at pH 2.5). The cosolvent approach was seemingly promising, provided that it had to overcome heavy precipitation observed by *in vitro* serial dilution method (see discussion in Section 4.3). Small amount of surfactants was then evaluated for the use in the cosolvent formulation in order to minimize or eliminate drug precipitation. The *in vitro* serial dilution method was used as a guiding tool for the screening process.

To begin with, the drug solubility was assessed in various cosolvent(s) vehicles, and the common vehicle (10% EtOH + 40% PEG400 in 0.1 M citrate buffer, pH 3.5) was identified as the major cosolvent system for further testing with surfactants. The compound has a solubility of 2.3 mg/ml in the above vehicle, but even a concentration at 1.0 mg/ml would observe heavy precipitation upon dilution with ISPB (isotonic Sorensen's phosphate buffer, pH 7.4), a buffer mimicking blood physiological dilution in the *in vitro* serial dilution model. A variety of surfactants were evaluated at 0.5%–1% (w/v) including Tween 80, Cremophor EL, Pluronic F68, Brij 97, Solutol HS-15, etc. The experiments were conducted as such: prepare the vehicle, i.e. cosolvents with different surfactants, respectively; dissolve the drug compound 2.1–2.2 mg/ml; dilute the formulation with ISPB (as specified in Section 4.3). After >25 rounds of testing (different surfactants, and different concentrations), a formulation with 2.2 mg/ml that would not precipitate upon mixing with ISPB was emerged. The final composition in the formulation: 2.2mg/ml drug in 10% EtOH + 40% PEG400 + 0.5% Pluronic F68 in 0.1 M citrate. The formulation has a pH at 3.3.

5.4. Case 4: SMEDDS screening and development

The compound is a preclinical lead. The study was to develop a lipid-based formulation for oral dosing in rats for PK study. The compound was poorly water-soluble (2.8 μg/ml), and poorly bioavailable with both suspension and solution formulations. Previous PK studies using micronized suspension generated a bioavailability as 3% in rat, and 5% in mouse. The Caco-2 data was not available due to the low solubility. It is likely that the compound is a Class IV. After initial formulation screening, it was decided to develop a microemulsion or a self-microemulsifying formulation to improve bioavailability. The screening procedure started with a selection of 10 oils and 10 surfactants. Oils were mostly structured-lipids includ-

ing Miglyol 812, Capmul MCM, Captex355, etc. Surfactants included Tween 80, Cremophor EL, Solutol HS-15, Pluronic F68, Acconon CC-6, Gelucire 44/14, etc. Out of these combinations, a few compatible oil-surfactants were selected for pseudo ternary phase diagram construction. Evaluation on these diagrams identified a promising system with 30% surfactant (a single surfactant) and 70% oil (two structured-lipids at 1/1 ratio): in the phase diagram, there are small regions of gel or semi-gel, but large region of microemulsion. Most importantly, dilution curve from the starting point (30% surfactant and 70% oils) falls in the microemulsion region. Test on the drug loading at 10 mg/ml onto the mixture of 30% surfactant and 70% oils found that the system remained largely unchanged in the phase diagram. This formulation or preconcentrate (10 mg/ml drug in 30% surfactant and 70% lipids) was then diluted with simulated GI fluids, and generated fine microemulsions with particle sizes at 32–37 nm. The final formulation was slightly modified for ease in transferring and administration: 10mg/ml drug, 65% oils, 25% surfactant, and 10% water.

6. Concluding comments and future perspectives

The importance of early formulations should never be underestimated. It enables the selection and optimization of drug compounds at different stages, mostly preclinical. Strong support from early formulations provides sound background for drug compounds' fair and speedy evaluation.

This study reviewed a broad scope of early formulations, relating to both basic aspect and development aspect. In basic aspect, it went in length on all major dosage forms and solubility enhancement techniques: principles, examples, and relevance to early formulations. With more and more drug compounds generated being poorly water-soluble and poorly bioavailable, the need for significant effort and approaches in early formulations are ever increasing.

The study shows that, despite the diversity and complexity, there are general principles that one can follow in developing early formulations. After reviewing a large volume of literatures, we present our understandings on strategies and approaches, which include the following:

(a) Formulation development schemes for oral (Fig. 1) and for i.v. (Fig. 2).
(b) Recommended use range for oral and i.v. early formulations (Table 4).
(c) The need, the experimental procedure, and the criteria for evaluating formulation precipitation potential upon dosing for solution formulations containing cosolvents and/or pH control.
(d) The importance, and the experimental procedure, for kinetic solubility.

Basic aspects remain just basic: a good understanding of the drug compound's physical chemical properties and of the request and limitation of formulation options is of critical importance. For most of the formulations, *in vivo* exposure is the driving force, but it has to be achieved accompanying dose accuracy and vehicle biocompatibility. It is ideal that none of the three components are compromised.

For solution formulations, solubility enhancement is the key for formulation selection and optimization. The pH adjustment, cosolvents, cyclodextrins, surfactants are still the mainstay in the development work. Special attention needs to be paid to the combined use of pH with cosolvents or cyclodextrins. Kinetic solubility is an effective tool in solubility and excipients screening and selection, and should be taken good advantage of. *In vitro* serial dilution is an effective tool especially for i.v. formulation.

Micronized or milled suspension can be used more frequently as an alternative option when some or all of the followings all present: conventional formulations inappropriate, high dose required, limited time available for extensive development work on novel formulations.

Novel formulations are reserved for formulations when conventional ones are inappropriate in addressing the need for *in vivo* exposure. Many novel formulations especially microemulsion, solid dispersion and nanosuspension are developable for the small batch (<10 g or ml) in a reasonably short time-frame. Formulations of this nature often prove to be critical in advancing the project relating to the early formulation. In general, there is a sense of relief in using these novel formulations, because most of the work (in PK) is for proof-of-concept, and hence, there is no immediate demand on formulation stability profiles (or there is, but at a later time). Microemulsion can be effective to BCS (biopharmaceutical classification system) Class II and IV compounds, while solid dispersion and nanosuspension are effective to Class II compounds. Solid dispersion is also known for its capacity in high payload in the dosage form. The fact that more and more drug compounds being poorly water-soluble/poorly bioavailable makes more room for novel formulations be developed, evaluated and used.

On maximum tolerable dose for excipient(s): literature on both commercial products (especially parenteral products) and toxicology data (LD_{50}) are useful source of information and should be taken good advantage of.

Establishing an in-house database may provide valuable support to early formulations in the long run. The database may contain information on drug (physical chemical and biopharmaceutical properties), formulation (composition, dosage form), application (efficacy or PK or toxicology, dose, route, animal species), and most importantly, study outcome/adverse effects on animals. The database keeps track of excipients/vehicles used in certain animal species/route/dose and adverse effects. The database may be established according to the area of therapeutic programs such as oncology, thrombosis, rheumatoid arthritis.

For the future trend in early formulations, it is believed that more effective excipients (with improved biocompatibility and safety profiles) will continue to be developed, which can be readily used in early formulation work. *In silico* evaluation will be increasingly used for early formulations, especially for drug compounds at discovery stage (discovery leads). It is also believed that, in due time, automation with systematic approach will take most of early formulation development work. In fact, many basic physical chemical properties can be measured such as solubility (especially kinetic solubility). The *in vitro* serial

dilution method can also be quickly automated for formulation selection and optimization. However, it is believed that the automation take-over is not imminent due to the complexity of the development work, especially for novel formulations.

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