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Review Article

Preclinical Formulations: Insight, Strategies, and Practical Considerations

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Abstract. A lot of resources and efforts have been directed to synthesizing potentially useful new chemical entities (NCEs) by pharmaceutical scientists globally. Detailed physicochemical characterization of NCEs in an industrial setup begins almost simultaneously with preclinical testing. Most NCEs possess poor water solubility posing bioavailability issues during initial preclinical screening, sometimes resulting in dropping out of an NCE with promising therapeutic activity. Selection of right formulation approach for an NCE, based on its physicochemical properties, can aid in improving its solubility-related absorption and bioavailability issues. The review focuses on preclinical formulations stressing upon different preclinical formulation strategies and deciphers the understanding of formulation approaches that could be employed. It also provides detailed information related to a vast pool of excipients available today, which is of immense help in designing preclinical formulations. Few examples mentioned, throw light on key aspects of preclinical formulation development. The review will serve as an important guide for selecting the right strategy to improve bioavailability of NCEs for academic as well as industrial formulation scientists.

KEY WORDS: biopharmaceutical classification system (BCS); oral absorption; oral bioavailability; permeation enhancers; preformulation.

INTRODUCTION

In the development of new chemical entities (NCEs) in the pharmaceutical industry, there is a timeline segment after the discovery of a potential NCE hit and before the start of human clinical studies, where a formulator is called upon to deliver formulations for various studies in preclinical (animal) species. In conventional pharmaceutical industry language, these formulations have come to be termed preclinical formulations (1,2). An example may be a simple suspension formulation of the NCE to be dosed via an oral gavage to mice for an efficacy study. Another example may be a cosolvent-based solution formulation of the NCE to be administered orally to rats in a pharmacokinetic study. Yet another example may be an intravenous injection of the NCE to beagle dogs for a toxicity (safety assessment) study (1,3). Although this work is primarily limited to this interval between synthesis by the medicinal chemist and first-in-human (FIH) phase I clinical study, formulations for nonclinical use are also requested during the post-FIH development phases and have come to be categorized as preclinical for convenience, in that they are not intended to be administered to humans. The current paper summarizes literature and non-literature data and our own experience regarding preclinical formulations of small molecule NCEs. This review is written to aid formulators who are called upon to design and develop preclinical formulations.

The role of a preclinical formulator is of utmost importance as it requires a thorough understanding of many aspects of drug development process. Apart from the knowledge of various formulation parameters, a preclinical formulator should have an understanding of toxicology, pharmacokinetics, analytical techniques, and chemical and physical characterization. As the NCE is still in its development phase, not much is known about its physicochemical properties. A good preclinical formulator can determine the physicochemical properties using advanced thermal and mechanical analysis techniques. A good physicochemical characterization by a formulator can provide an understanding on the physical stability of the NCE and thus interact with a chemist in lead optimization. A formulator should work very closely with the analytical scientist to identify stability issues that can derail or save an NCE at a later stage. A formulator must understand the needs of a toxicologist or a pharmacokineticist with

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ABBREVIATION: NCE, New chemical entity; FIH, first in human; API, active pharmaceutical ingredient; GLPs, good laboratory practices; GIT, gastrointestinal tract; SNEDDs, self nano-emulsifying drug delivery systems; CTAB, cetyl trimethyl ammonium bromide; CPC, cetyl pyridinium chloride; LCTs, long-chain triglycerides; MCTs, medium-chain triglycerides; SCTs, short-chain triglycerides; DMPK, drug metabolism and pharmacokinetics; SOP, standard operating procedure; DSC, differential scanning calorimetry; TGA, thermogravimetric analysis.

respect to the amount of dose that needs to be administered. The other information that a formulator should have includes requirement of drug administration to species under investigation and safety of components used in formulation. A preclinical formulator plays a very crucial and a central role in the run up of the NCE from the wet chemistry lab to the preclinical testing phase.

The development and supply of preclinical formulations present significant challenges for the formulator as follows: (a) the low solubility of most of today's NCE molecules requires an understanding of various solubilization approaches that can be incorporated in the formulation strategy; (b) the choice of excipients needs to be made with careful thought with regard to animal-specific toxicity and impact on NCE performance; (c) the volumes that can be administered are small considering the fact that the animal species are often rodents, although dogs and monkeys can consume larger doses, volumes, and even human dosage forms (e.g., capsules); and (d) very little time is available for development (4,5). A key objective in preclinical studies is to maximize exposure or efficacy by presenting the highest possible number of molecules of the NCE at the site of action. This is often achieved by the intelligent use of physicochemical principles in designing the formulation composition and presentation.

In this review, we present (a) various formulation strategies commonly employed in preclinical formulations; (b) excipients commonly used in these formulations; (c) details of each type of formulation, how it is developed, and how it is prepared; (d) logistics of delivering, using, and testing preclinical formulations; and (e) some notes on preclinical formulations in a good laboratory practice (GLP) setting. In this review, our focus is on the formulation and not on the animal species, the animal models, drug pharmacokinetics, or the bioanalysis that are indeed all part of preclinical animal evaluations but outside the scope that we have defined for this review.

PREFORMULATION

The FDA defines an NCE as a drug that contains no active moiety approved by FDA (6). Before designing an appropriate formulation for *in vivo* studies, it is important to know and understand these physicochemical properties. Often, the medicinal chemistry and early biology efforts focus on finding an efficacious molecule, and the determination of a physicochemical profile can take a back seat. Hence, a first step when presented with a molecule for preclinical formulation development must be an accurate characterization of physicochemical properties (like physical form, melting point, pKa, Log P, and aqueous solubility). Data thus generated should be shared with the medicinal chemist who can incorporate these learnings in any structure optimization efforts geared towards making the molecule more drug-like.

A real constraint at this stage of development is the limited amount of active pharmaceutical ingredient (API) available. Often, the medicinal chemist can only share a few milligrams. A preformulation laboratory must develop expertise, experience, and innovative approaches to generate meaningful and required data with the minimum amount of API available (7). For instance, we have optimized the procedure and perform important physicochemical characterization using only about 25 mg of the API (8). Additionally, we generate this data in a short time (a maximum of 2 days for a solution state study and a maximum of 8 days for a preliminary preformulation battery report) (8). With this, we get useful information on solubility, log P, solution state stability, melting point, and morphology to aid the formulator in designing a formulation. In our experience, it is important to characterize each batch of API received from the medicinal chemist minimally via differential scanning calorimetry and X-ray powder diffraction pattern, these requiring only about 10 mg of API. This database helps to ensure that the API of the same morphology gets used in efficacy, pharmacokinetics, and toxicity studies leading to appropriate comparisons and rational data evaluation or at least to help explain any differences, were they to arise.

Though not a stringent requirement for an R&D preformulation lab, compliance to GLP is always appreciated. Without adding undue bureaucracy (and resultant loss of time), it is best to instill a practice of thorough documentation including procedures followed in every experiment, record of API samples and batches used, results obtained, interpretations made, technical reports, and rationalized recommendations to both the medicinal chemist and the formulator. It is always best to assume that all data and documentation is auditable and to practice procedures that are aligned with such an assumption. Figure 1 displays a schematic representation for an NCE in its usual preclinical development.

RELATION OF BIOAVAILABILITY AND PHYSICOCHEMICAL PROPERTIES

The FDA has given guidance on the biopharmaceutical classification system (BCS) with regards to its purpose and goal (9). The biopharmaceutical classification system is classified into four major categories as described in Fig. 2 (10). The aim of a formulation scientist is to emulate by formulation approach BCS class I properties to BCS class II and BCS class IV drugs. The thoughtful selection of formulation excipient, delivery system, and thorough understanding of physicochemical parameters can help in improving the bioavailability of NCE that belong to BCS class II and BCS class IV categories. The various formulation approaches are discussed in detail in further sections.

SOLUBILITY STUDIES

Determination of solubility of the NCE in various media is an important step in determining the right formulation approach for NCE development (11). Solubility studies are usually carried out in the pH range 1.2 to 7.4 using different buffer compositions. The buffers commonly used are hydrochloric acid buffer pH 1.2, acetate buffer pH 4.5, phosphate buffer pH 6.8, and phosphate buffer pH 7.4. Additionally, solubility of the NCE is also determined in different biorelevant media like FaSSIF and FeSSIF. Kinetic solubility is also determined to understand the extent to which a compound can precipitate. In general terms, equilibrium/thermodynamic solubility determines the solubility of the crystalline form of drug, whereas the kinetic stability determines the solubility of the amorphous form of drug and hence most of the times the kinetic stability overestimates the solubility as compared to thermodynamic solubility (12). For determination of equilibrium/thermodynamic solubility, minimal

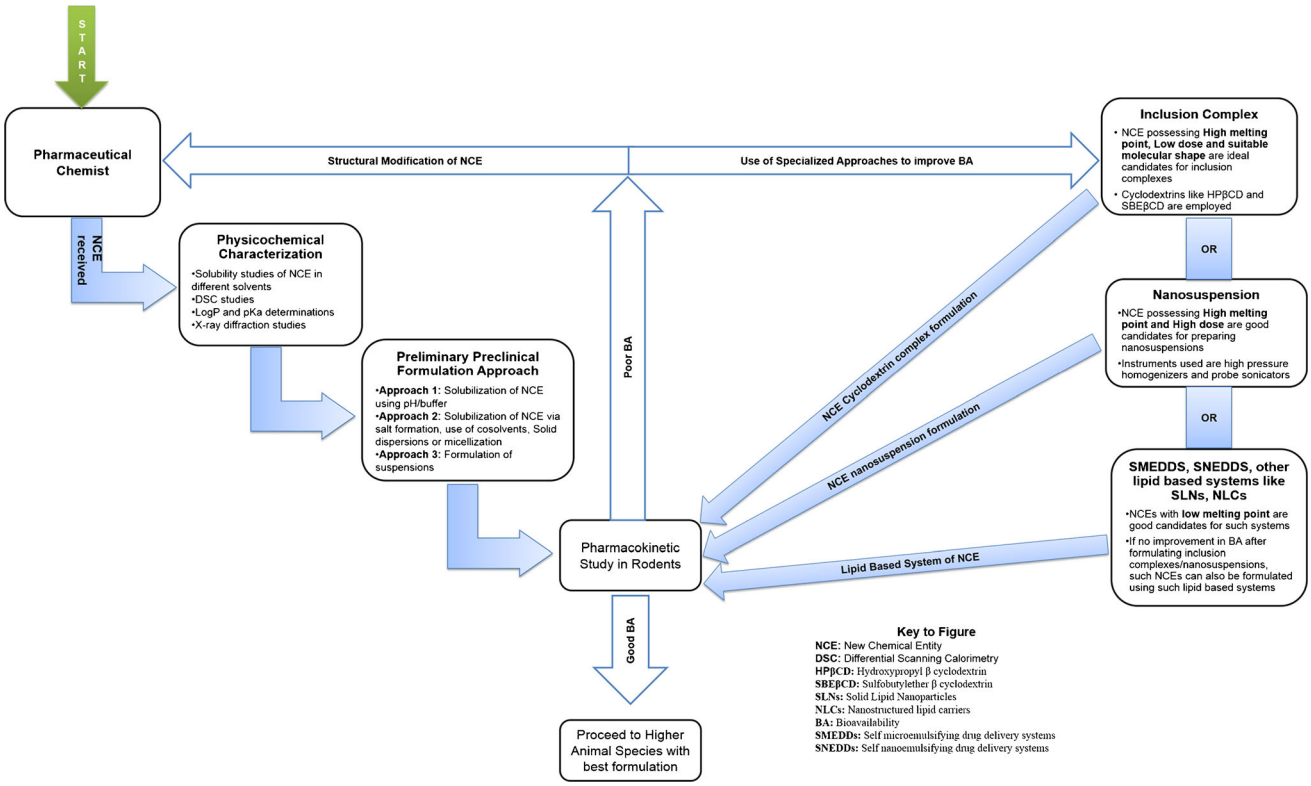

Fig. 1. A schematic of a decision tree for a new chemical entity based on various physicochemical properties

quantity of NCE (usually 1 mg) is suspended in the media (usually 1 mL), and the suspension is kept in a temperature-controlled water bath at 37°C for 24 h. After reaching equilibrium, the media is separated from the undissolved NCE either by centrifugation or by membrane filtration and analyzed for NCE by suitable analytical technique.

PERMEABILITY STUDIES

One important consideration in determining the right NCE candidate to progress further to preclinical animal testing is determining the apparent permeability coefficient using the Caco-2 monolayer permeability technique (13). Caco-2 is a heterogeneous human epithelial colorectal adenocarcinoma cells that possess P-glycoprotein efflux transporters, various enzymes (such as peptidases and esterases), microvilli, and tight cellular junctions mimicking the intestine. Caco-2 permeability assays can be used to assess the permeability of pure NCE or of the NCE in formulation. This is an *in vitro* diagnostic tool that can tell the formulation scientist about improvement of permeability of NCE across the Caco-2 cell monolayer. The advantage of Caco-2 assay is the ability to screen large number of samples in a short period of time with reproducible results. Different formulations of the NCE are screened by Caco-2 assay and a few successful formulations are further tested for *in vivo* pharmacokinetic study. Thus

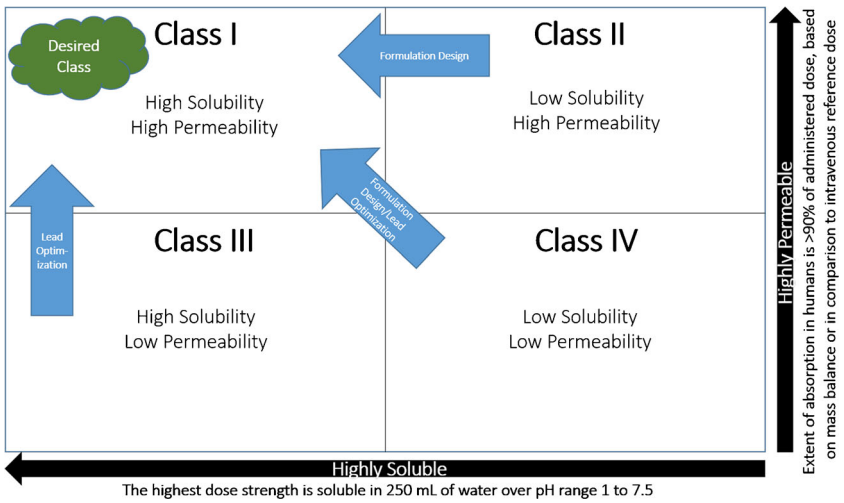

Fig. 2. The biopharmaceutical classification system (9,10)

Caco-2 permeability assays become a very useful tool to study improvement in permeation of drugs belonging to BCS class III and IV (possessing poor lipophilicity and consequently poor permeability) and/or for drugs that undergo excessive *P*-glycoprotein efflux and/or for drugs extensively metabolized by enterocytic enzymes. However, for estimation of only improvement in permeability, even artificial membranes can be used for the study as is done in parallel artificial membrane permeability assay (PAMPA) (14). In simple words, PAMPA assay is useful for drugs absorbed by passive diffusion and not by active transport, unlike Caco-2 cell lines that help in the evaluation of both passive and active uptake.

FORMULATION APPROACHES

Once the required minimum physicochemical characterization is done, the work of developing the formulation begins in earnest. A few scenarios encountered in early preclinical evaluation of NCE to improve oral bioavailability are depicted in Fig. 3. This approach can take either the conventional or the specialized route.

Conventional Approaches

Solutions are most preferred for preclinical evaluation because the NCE is presented in a state ready for absorption. It involves the preparation of monophasic aqueous mixtures of NCEs in water with or without aid of energy (in form of sonic waves, heat, or vortexing) (1). For bioavailability determinations, intravenous administration of an NCE requires the drug to be either solubilized or be presented as fine dispersion of particle size <3 microns thus not resulting in capillary blockade (15). Cosolvents such as ethanol, propylene glycol, and polyethylene glycols can be employed to formulate an NCE with low intrinsic aqueous solubility, in a solution dosage form. With solutions, a major concern is solubility (API precipitation) and solution stability. Data generated during the physicochemical characterization (solubility and stability as a function of concentration, temperature, ionic strength, buffer strength, etc.) is useful in making rational choices. Sometimes, the use of pH modifiers and taste modifiers may be warranted.

Suspensions may be the formulation option for dose determination and toxicity studies where high doses are to be administered orally to animals as the concentrations required for such high doses invariably exceed equilibrium solubility values of these molecules (16). Doses as high as 100 times the dose that is effective in 50% of the animals studied (ED_{50}) or as per FDA recommendations of 2 g/kg are to be evaluated in case where the NCE under investigation does not display/exhibit adverse effects at preclinical stages before proceeding to phase I clinical studies in humans (17). With suspensions, a major concern is the nonuniformity of dose due to time-dependent settling and agglomeration of particles. This is best overcome via the use of good suspending and deflocculating agents to ensure that the solid particles can be redispersed uniformly throughout the volume and remain uniformly suspended for a sufficient time after shaking. To ensure drug uniformity and ease of redispersion, surfactants (0.1–1%) and viscosity modifiers are employed in formulating suspensions. Generally, batches as small as 2–10 mL are prepared in vials or beakers precalibrated to a required volume. Initially, a paste of NCE is formed employing a continuous phase (either in the mixing vessel or mortar) to ensure complete wetting of particles and then the volume is made up to a precalibrated volume with continuous stirring. Probe homogenization may be employed as necessary for breaking particle agglomerates. A new method of preparing well-milled and highly homogenous suspension using small hard zirconium balls was reported by Niwa *et al.* (18).

Salts are known to improve solubility of molecules several fold. This can enhance availability of solubilized drug in the gastrointestinal tract (GIT) and thus the flux across the gut and improved bioavailability (the percentage of the drug that appears in the blood plasma). For polar, hydrophobic, or

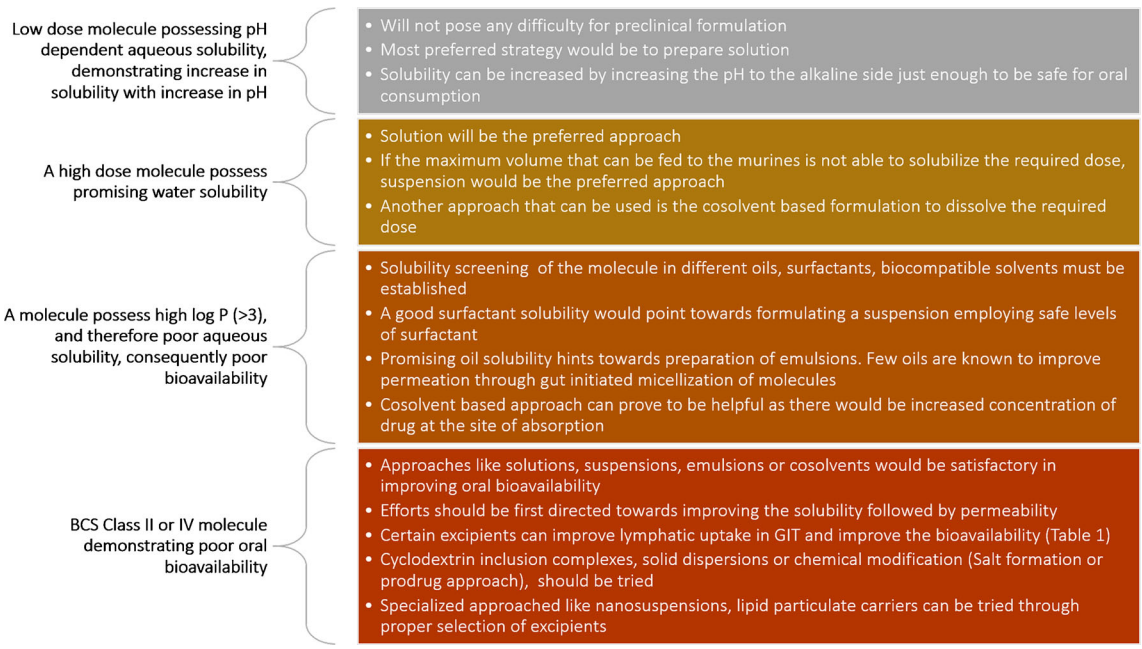

Fig. 3. Preclinical formulation scenarios encountered for improving oral bioavailability

lipophilic molecules possessing ionizable groups, salt formation is a feasible approach to improve bioavailability. While most salt forms improve nonequilibrium solubility and hence bioavailability of the parent compound, some fatty acid-based salt forms such as laurate and stearate may reduce the solubility, and yet increase bioavailability due to increased lymphatic uptake leading to prolonged blood levels (19). Many pharmaceutical drugs on the market are salts employing counterions such as hydrochloride, mesylate, citrate, acetate, tosylate, maleate, chloride, bromide, etc. (19–22). The main concern for salt formation is salt disproportion which is reversion of the salt to its unionized form. Salt disproportionation is an undesirable event as it changes the dissolution rate and changes in physical and chemical stability. Apart from the type of salt and aqueous solubility of salt, excipients characteristics like solubility, physical state, surface area, and its contribution to pH can influence salt disproportionation. If an excipient has the capability to alter the microenvironment pH, then it can lead to salt disproportionation. There are various indicators for salt disproportionation like change in intrinsic solubility, loss in potency of a tablet containing the salt, change in tablet hardness and disintegration time, change in thermal behavior, and change in X-ray diffraction pattern (23). It is difficult to find out salt disproportionation using techniques like HPLC as both free form and salt of the drug behave similarly in buffered phases. But a thorough understanding about salt formation and the excipient can minimize the phenomenon of salt disproportionation. Raman spectroscopy is a technique that can distinguish between free form and salt form of the drug. Use of a high-throughput robotic instrument for salt screening with a 96-well plate station is recommended. The important consideration in a high throughput set up is the heating/cooling rate, incubation temperature, type of anti-solvent, rate of anti-solvent addition, rate of evaporation, mixing rate, and crystallization vessel design (24). All these process variables will help in evaluating the various crystalline forms, hydrates, solvates, and polymorphs of the NCE. The important things that are required to be given consideration for selecting the best salt form are described in Fig. 4 (25,26). It helps to screen several counterions with different crystallization solvents. Scale-up of potential salt hits (20 mg scale) is performed, and on the basis of important considerations (that includes solubility, crystallinity, etc.) as described in Fig. 4, a particular salt form is chosen and the process transferred to a chemist for gram scale manufacture (24). The various parameters that are varied to effect salt formation are heating rate, rate of anti-solvent addition, and rate of evaporation. It is recommended that one use only pharmaceutically acceptable salts for salt screening. Salt screening often runs in parallel with formulation development by alternate means.

Cosolvent approach is also widely used to formulate poorly soluble drugs. It involves judicious combination of different solvents to effect drug solubilization via a simple process of vortexing or sonication with or without the aid of heat. Various cosolvents used include different grades of polyethylene glycols like PEG 100, PEG 200, PEG 300, PEG 400, and PEG 600; propylene glycols; glycerol; diethylene glycol monoethyl ether; pyrrolidones; glycofurol; alcohol; Soluphor P; Pharmasolve; etc. (27–29). The primary concern of a cosolvent-based approach is the precipitation of solubilized drug upon dilution with aqueous fluids. It is possible to prepare cosolvent formulations of a few drugs that do not precipitate upon dilution using dilution curves, but a present day NCE would seldom give a formulator an opportunity to prepare rugged cosolvent formulations which would not precipitate upon dilution. This is a common phenomenon as cosolvent-based approach makes a supersaturated solution of the active and thus leads to precipitation upon contact with aqueous compartment. The important thing one should consider is the resultant size of the drug precipitated. If upon precipitation the size is in submicron range, there is still a possibility of better absorption from GIT and thus improved oral bioavailability. Apart from drug precipitation upon dilution, the factors that should be given due consideration are general toxicity of the cosolvent, tonicity with respect to biologic fluid, viscosity, taste, and stability of the formulation components other than drug (30). A combination of a cosolvent and a surfactant will only result in a slight change in solubility owing to decreased solubilization capacity of the surfactant micelles (31).

Lipid-based systems play an important role in the formulation of molecules with poor permeability (formally characterized as BCS class III and IV). These include emulsions and oily solutions and dispersions. An emulsion has an immiscible liquid dispersed in another immiscible liquid and stabilized by virtue of emulsifying agents. Oily solutions or dispersions on the other hand include drug solubilized or dispersed in a suitable oily vehicle.

Invariably, the best approach for initial preclinical studies for an NCE is preparing simple solutions or suspensions and evaluating pharmacokinetic parameters. This approach serves two purposes. Firstly, it gives an indication of whether the NCE can reach the therapeutic concentrations on the basis of its own physicochemical properties. Secondly, if the NCE does not reach the desired therapeutic concentrations, it gives a strong rationale to develop novel delivery systems which will aid it in reaching the required therapeutic concentrations.

Specialized Approaches

Cyclodextrins inherently possess good water solubility, especially the derivatives of β-cylodextrin (hydroxypropyl β-cyclodextrin and sulfobutyl ether β-cyclodextrin), owing to many hydroxyl groups present on their outer surface in combination with a hydrophobic interior cavity where a hydrophobic drug molecule can fit in. The inclusion complex of the NCE and cyclodextrin thus formed possesses better solubility than the drug molecule as it is governed by the solubility of the cyclodextrin. Various cyclodextrin-based products which are in market for different routes include as Mitozytrex™ (mitomycin and HPβCD) and Sporanox® (itraconazole and HPβCD) for i.v. administration and Ombeta (omeprazole and βCD) and Meiact (cephalosporin and βCD) for oral administration. The complexes can be formed by employing various techniques such as grinding, evaporation of solvent used to solubilize cyclodextrin and NCE, spray drying, or lyophilization of cyclodextrin solution containing NCE (32–36).

Dispersions present the API in a higher energy state thereby enhancing transient solubility. Methods employed to yield solid dispersions or solid solutions of NCEs include co-evaporates, ground dispersions, or hot melt extrudates of NCEs with hydrophilic matrix carriers like high molecular

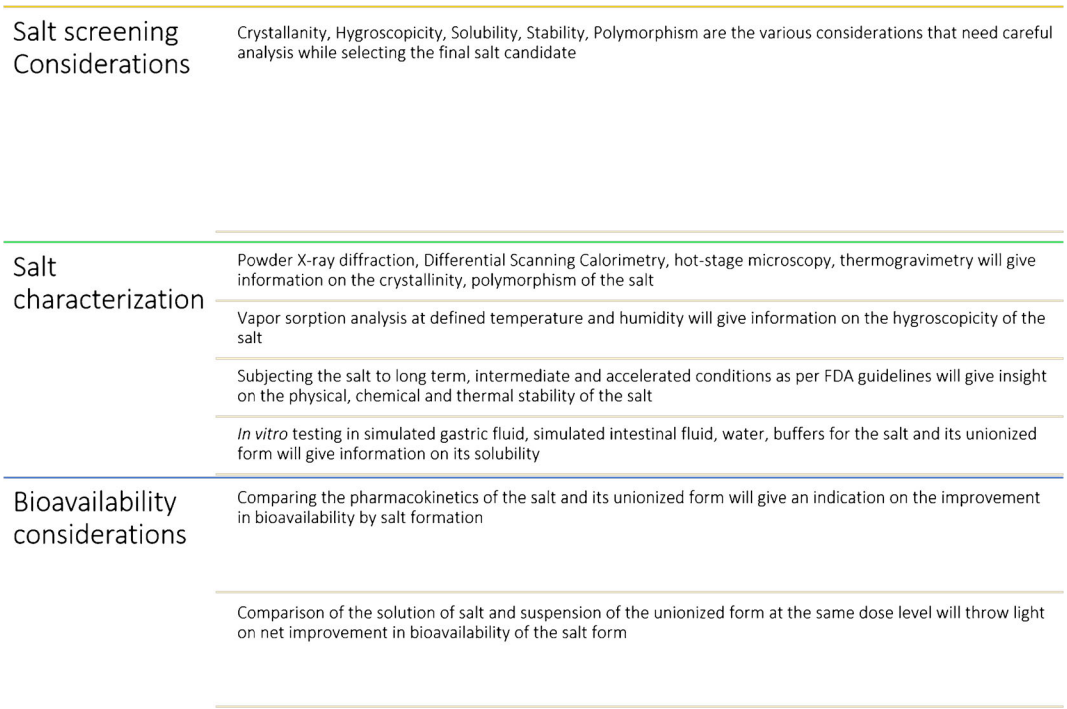

Fig. 4. Important considerations in selecting the final salt form (25,26)

weight polyethyleneglycols, polyvinyl alcohols, hydroxypropyl cellulose, polyethylene oxide, Eudragit E 100, Soluplus®, and Kollidon® VA 64 that can cause drug to either disperse uniformly or solubilize in hydrophilic carrier's matrix resulting in greater drug solubilization *in vivo* (37–42). One should give due consideration in design of solid dispersions of an NCE, as there is a tendency of NCE to crystallize out of the solid dispersion upon long-term storage which gets enhanced in the presence of moisture. Things that can effect crystallization of drug from solid dispersions include supersaturation of drug in polymer matrix, hygroscopicity, and temperature changes. A formulator should thermally characterize solid dispersions to understand the physical state of drug in the polymer matrix. Accelerated stability testing followed by thermal characterization like DSC, TGA, and hot-stage microscopy as well as vapor sorption studies of the solid dispersions can hint at the possibility of drug precipitation. Carrier to drug ratio, right choice of container closure system, limited exposure to humidity, and storage in cool environment are few measures that can be taken to retard drug crystallization from polymer matrix.

Nanosuspensions constitute another approach to ensure increase in drug dissolution rate due to increased surface area exposed to dissolution media. Nanosuspensions for various drugs belonging to the class of antiemetics, anticancer, and immunosuppressant administered by different routes have been prepared and have entered the market or are in different phases of clinical trials. They can be prepared by top-down techniques such as ball milling, high-pressure homogenization, or bottom-up techniques like nanoprecipitation effected by solvent diffusion or evaporation (43–49). Ball milling is performed using the milling media which can be composed of glass, zirconium oxide, or polystyrene resin along with water, drug, and stabilizer solution which are milled under controlled temperature and constant shear rate for up to 7 days. The advantage of ball milling is its ability to handle concentrated suspensions. High-pressure homogenization is a technique in which dispersion of drug, and stabilizer in water is passed through a very narrow orifice created between two ceramic beads. There is size reduction due to cavitation forces, shear force, and collision of particles. The operating pressure and the number of cycles both govern the final particle size of the nanosuspensions. In the bottom-up technique, like solvent diffusion or evaporation, a nonaqueous solution of drug is added to the aqueous stabilizer solution followed by high shear using a mechanical stirrer. Controlled evaporation of the nonaqueous solvent results in nanosuspensions. Surfactants like polysorbates like Tween 80, cremophors, poloxamers like Lutrol F68, Myrj, and Solutol HS 15 are employed for formulating nanosuspensions along with the aid of co-surfactants like Labrasol, Gelucires, PEG 100, PEG 200, PEG 400, PEG 600, and lecithin. Glycerol is sometimes used to adjust viscosity with concomitant advantage of adjusting osmolarity. Dispersion medium usually consists of aqueous phase. In-organics salts like NaCl and buffers are added for tonicity and pH adjustment, respectively, for i.v. preparations. Various nanosuspensions-based products are available in the market which include Rapamune® (sirolimus), Emend® (aprepitant), TriCor® (fenofibrate), Megace® ES (megestrol acetate), and Triglide™ (fenofibrate).

Nanoemulsions, by virtue of the nanosize of globules, offer greater surface area in comparison to their coarse emulsion counterparts, resulting in better contact with GIT membranes for better absorption. The nanosize of oil droplets may result from the use of suitable quantity of stabilizers and/or from energy given in form of high-pressure homogenization or sonication (50,51). Nanoemulsions have proven to possess

great potential to improve efficacy of drugs when administered via oral (52–55), topical (56), parenteral (57), nasal (58), as well as ocular route (59). To improve stability of nanoemulsions, self-nanoemulsifying drug delivery systems (SNEDDS) can be administered orally as preconcentrates which upon interaction with GI fluids will yield nanoemulsions and facilitate oral absorption (60). Today, a range of specialized stabilizers is available to ensure enhanced colloidal stability of these nanosystems. The oil phase for a SNEDDS consists of long-chain triglycerides like soyabean oil, corn oil, groundnut oil; medium-chain triglycerides like Capmul MCM, Miglyol 812, Miglyol 810, Capryol 90, Capmul MCM C8, and Imwitors. Surfactants employed are from the class of polysorbates like Tween 80, cremophors, poloxamers like Lutrol F68, Myrj, and Solutol HS 15 along with co-surfactants like Labrasol, Gelucires, PEG 100, PEG 200, PEG 400, PEG 600, and lecithin. Aqueous phase can be plain water or buffered water. Nanoemulsion-based formulations in the market include Limethason® (dexamethasone), Diprivan® (propofol), and Neoral® (cyclosporine).

Amorphous solids possess the advantage of better non-equilibrium solubility than their crystalline counterparts. Most techniques used to obtain amorphous solids include sudden change of process parameters to arrest molecules in a disorderly arrayed structure of higher energy than its crystalline counterpart. Approaches to generate amorphous form are spray-drying, hot melt extrusion, freeze-drying, and snap-freezing (quench cooling). The amorphous form thus generated can then be administered as such or as part of another dosage form.

EXCIPIENTS

An excipient is a pharmacologically inactive substance used to confer desirable properties to the formulation which delivers the drug molecule. Pharmaceutical regulations require that all ingredients in formulations, as well as their chemical decomposition products, be identified and guaranteed to be safe (61). Although this applies to clinical and marketed formulations, a similar "nontoxic-to-species" approach needs to be taken in preclinical studies also. For these reasons, excipients are only used when absolutely necessary and in the smallest amounts one can justify.

Ideally, if no excipient is used and the NCE is administered as is, one would have the least complicated "formulation." However, excipients need to be incorporated in the formulation for many valid reasons. In many cases, the NCE may not be easily administered and absorbed by the animal; in such cases, the NCE may be dissolved into or mixed with an excipient that renders it in a desirable form for absorption. In some cases, they may alter the intestinal permeability or in some cases they may participate in special absorption mechanisms. Excipients may be used to bulk up the formulation to provide bulk weight or volume convenient for administration and or for accurate dosage delivery, to aid in handling of the active substance during formulation manufacture (e.g., to aid flow or avoid segregation), to solubilize the NCE, to stabilize the NCE, to improve bioavailability of the NCE, etc. Excipients may also be added to render the active stable during preparation, storage, and administration. Excipients may also be added to keep particles segregated and suspended. Nanosizing, for instance, increases the surface energy of particles and their tendency to aggregate (62). Use of appropriate polymers can help prevent or minimize aggregation. It is apparent then that the formulation scientist must possess a detailed understanding of different excipients and their relevance to various formulation strategies employed. A detailed understanding of the correlation between physicochemical properties of the NCE and the physicochemical properties and functional roles of various excipients is needed. Additionally, the formulator must have access to a database on acceptable levels of each excipient for each administration route and animal species. Various classes of excipients are used in preclinical formulations including, but not limited to polymers, surfactants, oils, emulsifiers, solubilizers, inorganic salts, and cosolvents. Excipients commonly employed for preclinical formulations are listed in Table I outlining its chemical composition, physical state, HLB value, LD_{50} value, route of administration, and its use. The uses of various excipients in actual preclinical formulation are listed in Table II.

The preferred approach for preliminary preclinical evaluation of NCEs consists of preparing a simple aqueous solution formulation. This allows for ease of administration, uniformity of content, and ease of manufacture. However, as mentioned earlier, poor aqueous solubility of majority of NCEs in development pipelines often necessitates the use of alternate strategies that includes formulating cosolvent-based systems, suspensions, emulsions, etc. Besides, poor aqueous solubility results in poor mass transfer across the gut wall and consequently low oral bioavailability. This often necessitates other formulation strategies such as solubilization techniques (solid dispersions and solutions, cyclodextrin inclusion complexes, salt formation, etc.), nanosuspensions, and nanoemulsions to improve oral bioavailability of a particular molecule. This helps to prevent a worthy NCE from getting eliminated from further development due to absorption, distribution, metabolism, and elimination issues. When preparing an aqueous or a nonaqueous simple solution, the formulator needs to ensure that a low-solubility polymorph is not precipitating out or the salt of the NCE does not disproportionate to the free form which, in turn, precipitates out, and that the product retains stability of the NCE for at least the duration from the time of manufacture to the time of administration under the conditions that the formulation will be held, and that ease of administration to the species under consideration is given due importance.

The next preferred formulation approach is a simple suspension. Considering the low solubility of most NCEs, in our laboratory, suspensions have been tested more than any other formulation. Celluloses, e.g., methyl cellulose, sodium carboxymethyl cellulose, and hydroxypropyl methyl cellulose or hypromellose are safe, inert, and commonly employed suspending agents for preparing suspension of NCEs for early preclinical evaluation. They are often used in combination with other nonionic surfactants that are categorized as polysorbates, cremophors, poloxamers, and so on. While most of these nonionic surfactants are safe at concentrations used (Table I), some cationic surfactants such as cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC) can also be used, but with safety constraints. Surfactants are important components of suspensions and emulsions. Many surfactants have been reported in literature to improve bioavailability of NCEs

Table I. Excipients Commonly Employed for Preclinical Formulations of NCEs

Name	Composition	Form[a]	HLB	LD_{50}	Route[b]	Use
Capryol™90 (63)	Propylene glycol monocaprylate (type II)	L	6.0	[c]	O, T	Bioavailability enhancer, solubilizer
Capryol PGMC (64)	Propylene glycol monocaprylate (type I)	L	5.0	[c]	O, T	Bioavailability enhancer, solubilizer
Lauroglycol™90 (65)	Propylene glycol monolaurate (type II)	L	5.0	Oral, Rat, >2 g/kg	O, T	Bioavailability enhancer
Lauroglycol™ FCC (66)	Propylene glycol monolaurate (type I)	L	4.0	Oral, Rat, 2 g/kg	O, T	Bioavailability enhancer
Labrafac™ PG (67)	Propylene glycol dicaprylocaprate	L	2.0	[c]	O, T	Bioavailability enhancer
Labrafil® M1944CS (68)	Oleoyl polyoxyl-6 glycerides	L	4.0	Oral, rat, >20 g/kg	O, T	Reduced hepatic metabolism due to increased lymphatic uptake
Labrafil®M 2125CS (69)	Linoleoyl polyoxyl-6 glycerides	L	4.0	[c]	O, T	Reduced hepatic metabolism due to increased lymphatic uptake
Labrafil® M2130CS (70)	Lauroyl polyoxyl-6 glycerides	SS	4.0	Oral, Rat, >2 g/kg	O, T	Better solubilization of APIs
Capmul® MCM (71)	Medium chain mono- and diglyceride of C_8 and C_{10} chain	L	5.0–6.0	Oral, rat, >5 g/kg	O, T	Emulsifier and lipophilic surfactant, Improves permeation and consequently oral bioavailability
Miglyol 810 and 812 (72)	Caprylic/capric triglyceride	L	–	Oral, rat, >5 g/kg i.p., rat, >8 g/kg	O, T, Pa	Absorption promoter in GIT and when used topically, parenteral nutrition, solvent for certain APIs
Lecithin (PL 90)	Diacyl glycerol phosphatidylcholine, consists of long-chain saturated and unsaturated fatty acids	S	4.0–9.0	GRAS (73)	O, T, Pa	Emulsifier and lipophilic surfactant, improves permeation and consequently oral bioavailability
Tween 80/Polysorbate 80 (73)	Polyoxyethylene 20 sorbitan monooleate	L	15.0	Oral, Rat, 25 g/kg i.v., rat, 1.8 g/kg i.p., rat, 6.8 g/kg	O, T, Pa	Emulsifying, wetting and dispersing agent
Tween 20/polysorbate 20 (73)	Polyoxyethylene 20 sorbitan monolaurate	L	16.7	Oral, rat, 37 g/kg i.v., mouse, 1.42 g/kg	O, T, Pa	Emulsifying, wetting and dispersing agent
Lutrol® F 68 (74)	Synthetic copolymers of ethylene oxide and propylene oxide	SG	>24.0	Oral, rat, >15 g/kg dermal, rabbit, >20 g/kg (75)	O, T, Pa	Emulsifying and surface active agent
Lutrol® F 127 (74)	Synthetic copolymers of ethylene oxide and propylene oxide	SG	18.0–23.0	Oral, rat, >10 g/kg dermal, rabbit, >5 g/kg (76)	O, T, Pa	Emulsifying and surface active agent; Capable of forming thermoreversible gels
Labrasol® (77)	Caprylocaproyl macrogol-8 glycerides	L	14.0	Oral, rat, >22 g/kg (78)	O, T, Pa	Bioavailability and permeation enhancer, emulsifier and surfactant
Geluciore® 44/14 (79)	Lauroyl macrogol-32 glycerides	SS	14.0	Oral, rat, >2 g/kg (80)	O	Surfactant, solubilizer, wetting agent, bioavailability enhancer
Geluciore® 50/13 (81)	Stearoyl macrogol-32 glycerides	SS	13.0	Oral, rat, >20 g/kg (82)	O	Surfactant, solubilizer, wetting agent, bioavailability enhancer
Vitamin E-TPGS (83)	D-alpha tocopheryl polyethylene glycol 1000 succinate	P	13.0	Oral, rat, >7 g/kg dermal, rat, >2 g/kg	O, T, Op	Emulsifier, drug solubilizer, absorption enhancer, vehicle for lipid-based drug delivery, source of natural vitamin E
Solutol® HS 15 (84)	Polyoxyl 15 hydroxy stearate	SS	14.0–16.0	Oral, rat, >20.6 g/kg (85)	O, Pa	Emulsifying, wetting and dispersing agent
Cremophor® EL, ELP (86)	Polyoxyethylene 35 castor oil	L	12.0–14.0	Oral, rat, >6.4 g/kg (87)	O, T, Pa	Emulsifying, wetting and dispersing agent
Cremophor® RH 40 (88)	Polyoxyl 40 hydrogenated castor oil	P/L	14.0–16.0	Oral, rat, >20 g/kg (89)	O, T, Pa	Emulsifying, wetting and dispersing agent
Transcutol® P and Transcutol® HP (90)	Diethylene glycol monoethyl ether	L	–	Oral, rat, >5 g/kg dermal, rat, 6 g/kg (91)	O, T	Solubilizing agent
Soluphor® P (92)	2-Pyrrolidone	L	–	Oral, rat, >2 g/kg dermal, rabbit, >2 g/kg (93)	O, T	Solubilizing agent

Pharmasolve V (73)	*N*-methylpyrrolidone	L	–	Oral, rat, 3.9 g/kg i.p., rat, 2.5 g/kg i.v., rat, 0.08 g/kg	O	Solubilizing agent
Glycofurol (73)	Tetrahydrofurfuryl alcohol polyethylene glycol ether	L	–	i.v., Mouse, 3.5 mL/kg	Pa	Solubilizing agent
PEG 200–400 (73)	Polyethylene glycols	L	–	PEG 200, oral, rat, 28 g/kg PEG 300, oral, rat, 27.5 g/kg PEG 400, oral, mouse, 28.9 g/kg	O, T, Pa, R	Solubilizing agent, improves permeation
Propylene glycol (73)	2-Hydroxypropanol	L	–		O, T, Pa	Solubilizing agent, improves permeability of skin and mucosa
Soluplus® (94)	Polyvinyl caprolactum polyvinyl acetate polyethylene glycol graft copolymer	SG	–	Oral, rat, >5 g/kg dermal, rat, >5 g/kg (95)	O	Solubilizing agent
LCTs (soyabean oil, corn oil, safflower oil, fish oil)	Glycerides of long chain fatty acids (C16–C20).	L	3.0–7.0	Soybean oil, i.v., rat, 16.5 g/kg (73) Safflower oil, i.p., mouse, >50 g/kg(73)	O, T, Pa	Useful for parenteral nutrition, useful for improving oral and topical permeation
Cyclodextrins (CD)	β-cyclodextrins and its derivatives (hydroxy propyl β-CD and sulfobutyl ether β-CD)	S	–	β-cyclodextrin, oral, rat, 18.8 g/kg(73) β-cyclodextrin, i.v., rat, 1 g/kg(73)	O, Pa	Excellent solubilizers for most lipophilic molecules

[a] *L* liquid, *S* solid, *P* paste, *SS* semisolid, *SG* solid granules
[b] *O* oral, *T* topical, *Pa* parenteral, *R* rectal, *Op* ophthalmic
[c] The product is not subject to classification according to the calculation method of the General EU Classification Guidelines for Preparations as issued in the latest version

through different mechanisms. The most common effect being their capability to alter GIT membrane fluidity and permeation characteristics. Besides, some of the surfactants such as polysorbates, cremophors, poloxamers, Solutol, and Gelucires are known to inhibit P-glycoprotein in GI mucosa reducing efflux of absorbed molecules, thus improving oral absorption (3,106). Taxol® is a clear, colorless to slightly yellow viscous nonaqueous solution of paclitaxel, a BCS class IV molecule, in Cremophor EL and dehydrated alcohol. The mechanism by which there is improved bioavailability is by Cremophor EL-induced micellization of paclitaxel followed by enhanced uptake (112) and reduced P-glycoprotein efflux of paclitaxel. An example of reduced P-glycoprotein efflux and use of drug micellization for improved bioavailability is Taxotere®, a formulation of docetaxel combined with polysorbate 80 and dehydrated alcohol. Docetaxel, a BCS class II drug, is a substrate for P-gp efflux leading to drug-resistant tumors (113). Addition of Tween 80 as a solubilizer, a P-glycoprotein inhibitor, is an added advantage as docetaxel is a known P-glycoprotein substrate (114). Another very good example of excipient-enhanced delivery system is Abraxane®, a paclitaxel albumin-bound nanoparticle for injectable suspension. This targeted strategy uses the albumin-activated gp60 receptor present on endothelial cells thereby increasing the concentration on the cytotoxic agent in the tumor cell.

Oils and lipids are also important excipients employed in preclinical studies. Lecithin is a well-known stabilizer used in preclinical suspensions and emulsions and is known for its potential to improve oral absorption by increasing micellization of drugs in GIT (110,115). Many long-chain triglycerides and medium-chain triglycerides used as either the oil component of emulsions or as stabilizers are known to exert similar effects. Long-chain triglycerides (LCTs) capable of boosting chylomicron production such as oleic acid, linoleic acid, and their derivatives are known to improve lymphatic uptake, thus reducing first-pass metabolism and improving overall bioavailability of drugs (116). Medium-chain triglycerides (MCTs) and some short-chain triglycerides (SCTs) have been exploited more by formulation scientists recently due to their greater solubilization potential in comparison to LCTs for most NCEs. This is common to almost all SCTs and MCTs available today including different grades of Capryol, Labrafil, Capmul, Miglyol, and Labrafac. Besides, some of the MCTs such as Capryol^{TM}90 (63) and Lauroglycol^{TM}90 (65) have been reported to inhibit certain enterocytic enzymes such as CYP3A4, thus reducing intestinal metabolism of most NCEs. Without exception, almost all MCTs are known to act as bioavailability enhancers of APIs by some mechanism or other. Their inclusion in negligible quantities in formulations is thought to augment absorption of poorly soluble or poorly permeable drugs through GI mucosa.

Physicochemical properties of stabilizers, such as their HLB values or fatty acid type and content are also useful in preparing formulations with better colloidal stability, especially for nanosystems. For instance, HLB values of a few excipients such as Labrasol (77), Gelucires 44/14 (79), and Vitamin E TPGS (83) is 13–14, suggesting their amphiphilic nature which makes them good solubilizers for almost all NCEs with varying lipophilicities. Such excipients are also widely reported for their potential to improve oral absorption.

While selecting excipients and their concentrations to be used in different formulations, it is important to know their

Table II. Database of Commonly Used Ingredients in Pre-clinical/Clinical Formulations

Excipient	Amount of excipients used (mg/kg)[a,b]	Route	Murine species	Reference
Vitamin E	75.8	i.v	C57BL/6 mice, male, 18–20 g	(96)
PEG-DSPE	75.8			
Cholesterol	75.8			
Gelucire 44/14	746.7	Oral	SD rat, male, 280–350 g	(97)
Hydrogenated castor oil 60	320.0			
Sodium dodecyl sulfate	0.4			
Tricaproin (oil)	8,000.0	i.p	ICR mice, 20–25 g	(98)
Egg PC	3,200.0			
Tween 80	2,400.0			
Tricaproin (oil)	6,000.0	i.p	ICR mice, 20–25 g	(98)
Tricaprylin (oil)	2,000.0			
Egg PC	3,200.0			
Tween 80	2,400.0			
Tricaproin (oil)	4,000.0	i.p	ICR mice, 20–25 g	(98)
Tricaprylin (oil)	4,000.0			
Egg PC	3,200.0			
Tween 80	2,400.0			
Captex 355	28.3	Oral	Beagle dogs, male, 15–18 kg	(99)
Capmul MCM	14.1			
Cremophor EL	9.1			
Absolute Ethanol	6.1			
Captex 355	20.2	Oral	Beagle dogs, male, 15–18 kg	(99)
Capmul MCM	10.1			
Cremophor EL	21.2			
Absolute ethanol	6.1			
Soybean oil	17.6	Oral	Beagle Dogs, Male, 15–18 Kg	(109)
Maisine 35-1	17.6			
Cremophor EL	18.2			
Absolute ethanol	4.2			
Soybean oil	64.7	i.v.	Wistar rats, male, 210–260 g	(100)
Lecithin	12.9			
Glycerin	14.6			
Soybean oil	64.7	i.v.	Wistar rats, male, 210–260 g	(100)
Poloxamer 338/Pluronic F108	12.9			
Glycerin	14.6			
Soybean phosphatidyl choline	34.2	i.v.	Wistar rats, male, 210–260 g	(100)
Solutol HS15	23.9			
PEG 400	55.7			
Ethanol	23.9			
Miglyol 810N (MCT)	215.0			
Soybean oil	334.5	Oral and i.v	SD rat, male, 280–300 g	(101)
Egg lecithin	66.9			
Glycerol	83.3			
Tween 80	25.3	Intranasal	New Zealand white rabbits; 3–4 kg	(102)
Propylene glycol	25.3			
Ethanol	6.5			
Ethyl laurate	12.2			
Tween 80	13.3	Intranasal	New Zealand white rabbits; 3–4 kg	(102)
Propylene glycol	13.3			
Ethanol	13.3			
Ethyl laurate	8.6			
Vitamin E	526.3	i.v.	C57/BL6 mice; 18–20 g	(96)
PEG-DSPE	526.3			

Cholesterol Oleic acid	526.3 526.3			
Cremophor RH 40 Labrasol Ethyl oleate Dextran 40	32.0 8.0 68.6 114.3	Oral	New Zealand white rabbits; 3–4 kg	(103)
Cremophor EL Carbitol Capryol 90 Lauroglycol 90	7.1 7.1 9.5 9.5	Oral	Beagle dogs; 10.20 - 12.2 kg	(104)
Labrasol[c] Transcutol Labrafil 1944 CS	2 mL/kg/day 1 mL/kg/day 2 mL/kg/day	Oral	6-weeks-old wistar rats	(105)
Propylene glycol Ethanol Cremohphor EL	0.4 mL/kg 0.1 mL/kg 120.0	Oral	SD rats; 300 g	(106)
Propylene glycol Ethanol TPGS[d] (106)	0.4 mL/kg 0.1 mL/kg 120.0	Oral	SD rats; 300 g	(106)
Propylene glycol Ethanol Acconon E	0.4 mL/kg 0.1 mL/kg 120.0	Oral	SD rats; 300 g	(106)
Propylene glycol Ethanol Softigen 767	0.4 mL/kg 0.1 mL/kg 120.0	Oral	SD rats; 300 g	(106)
Propylene glycol Ethanol Inwitor 742	0.4 mL/kg 0.1 mL/kg 120.0	Oral	SD rats; 300 g	(106)
TPGS	120.0	Oral	SD rats; 300 g	(106)
Softigen 767[e] (106)	120.0	Oral	SD rats; 300 g	(106)
Transcutol P Tween 80 Labrafil M1944 CS	0.48 mL/kg 360.0 240.0	Oral	Swiss Albino mice; 20 g	(107)
SBEβCD	275.0	Oral	Swiss Albino mice; 20 g	(33)
Eudragit EPO Tween 20 Soluphor P	50.0 150.0 0.25 mL/kg	Oral	SD rats; 250 g	(108)
DDAB Soluphor P	13.33 0.13 mL/kg	Oral	SD rats; 250 g	(109)
DDAB Lecithin Transcutol P	222.0 372.0 1 mL/kg	Oral	C57/BL6 mice; 18–22 g	(110)
Precirol ATO 5[f] (111) Gelucire 50/3 Transcutol P	1041.6 333.0 1.25 mL/kg	Oral	C57/BL6 mice; 18–25 g	(111)

[a] The amount of excipients administered (in mg/kg) is calculated by dividing the amount of excipients administered per unit dose by the weight (in kg) of the murine species used
[b] This is not the LD_{50} value of the excipient
[c] The three ingredients Labrasol, Transcutol and Labrafil 1944 CS were evaluated for both the sexes for a total period of 4 weeks and no observable effect was reported
[d] Enhancement in absorption of API (digoxin) was observed
[e] Softigen 767 enhanced the absorption of API (celiprolol). It modified the paracellular passage by opening the tight epithelial junctions
[f] The animals were dosed on alternate days up to day 21

Table III. Formulation Types and Its Respective Characterization

Formulation type	Equipments/ apparatus required for preclinical studies	Excipients required[a]	1° Characterization[b,c]	2° Characterization[d,e,f]
Aqueous solution	Bath sonicator, vortexers, water bath	Cyclodextrins, pH modulators, surfactants	Physical stability like color, precipitation on storage	–
Cosolvents	Bath sonicator, vortexers, water bath	Pharmasolve, PEG, propylene glycols, transcutol P, surfactants	Physical stability like color, precipitation on storage	–
Suspension	Bath sonicator, vortexers, water bath, mortar and pestle, over head stirrer, homogenizer	Cellulose derivatives (*NaCMC*, *HPMC*, *HPC*), surfactants (*polysorbates*, *polaxamers*), viscosity modifiers (*glycerol*, *gums*)	Drug uniformity Ease of redispersion	Particle morphology using light microscope
Coarse emulsion	Bath sonicator, vortexers, water bath, mortar and pestle, over head stirrer, homogenizer	Oils (*MCTs*, *natural oils*), emulsifiers (*polysorbates*, *polaxamers*, *cremophor*)	Physical stability like creaming and cracking	Globule size using light microscope
Oily solution/ dispersion	Bath sonicator, vortexers, water bath, mortar and pestle, over head stirrer, homogenizer	Oils and their mixtures (*MCTs* and *natural oils*)	Drug uniformity	–
Solid dispersion	Rotary evaporator, water/paraffin bath, heating mantle, lab-scale hot melt extruder, lab-scale spray dryer, lab scale lyophilizer, mortar and pestle	Water-soluble polymers (*HPC*, *PVA*, *HPMC*, *alginates*, *high molecular weight PEGs*), amphipathic surfactants (*Gelucire*, *vitamin E TPGS*), inert adsorbents (*colloidal SiO_2*, *aluminum magnesium silicates*), cyclodextrins and other carbohydrates	Drug uniformity, phase solubility and solubility enhancement evaluation studies, physical stability	Solid-state characterization (*XRD*, *DSC*, *NMR*, *FTIR*, *hot stage microscopy*) Surface morphology (*light microscope and SEM*)
SNEDDS[g]	Vortexer, bath sonicator, water bath	Oils (*MCTs*, *Natural oils*), emulsifiers (*polysorbates*, *polaxamers*, *cremophor*) Co-emulsifiers (*Labrasol*, *transcutol P*)	Drug uniformity, particle size, physical stability, like phase separation	Surface morphology (*TEM*, *SEM*)
Nanosuspension	Rotary evaporator, probe sonicator, lab-scale high-pressure, homogenizer, vortexer	Surfactants (*polysorbates*, *polaxamers*, *cremophors*)	Particle size, colloidal stability	Surface morphology (*TEM*, *SEM*) Solid-state characterization (*DSC*, *XRD*)
Lipid-based nanodrug delivery systems	Water bath, vortexer, high-pressure homogenizer, probe sonicator	Solid lipids (*Glycery monostearate, Glyceryl behenate*), liquid lipids/oils (*MCTs*), surfactants (*polaxamers*, *polysorbates*, *cremophor*)	Particle size, entrapment efficiency, colloidal stability	Solid-state characterization (*DSC*, *XRD*) Surface morphology (*SEM*, *TEM*, *or CryoTEM*)

[a] *PEG* polyethylene glycol, *NaCMC* sodium carboxymethyl cellulose, *HPMC* hydroxy propyl methyl cellulose, *HPC* hydroxy propyl cellulose, *MCTs* medium-chain triglycerides, *PVA* polyvinyl alcohol

[b] Drug content/assay and short-term chemical stability (an indication of drug and formulation excipient compatibility) using a suitable HPLC method is common primary characterization for all the formulations intended for preclinical use

[c] 1° Characterization should be performed before the formulation is submitted for preclinical evaluation

[d] 2° Characterization can be performed after the formulation shows promise in initial preclinical evaluation

[e] Promising preclinical studies will necessitate the need for establishing long term chemical and physical stability of the formulation under investigation

[f] *XRD* X-ray diffraction, *DSC* differential scanning calorimetry, *SEM* scanning electron microscopy, *TEM* transmission electron microscopy, *NMR* nuclear magnetic resonance, *FTIR* Fourier transform infrared spectroscopy

[g] *SNEDDS* self-nanoemulsifying drug delivery systems

LD_{50} values reported in different animals, and accordingly use them within their safety limits. We generally target the use of excipients at a tenth of their LD_{50}. Conducting a thorough literature survey about their toxicity and safety profile during preclinical studies ensures minimal complications during clinical trials.

Data presented in the literature on the type of excipients used, its concentration, and the amount of excipient administered in a single dose is tabulated below (Table II). The type and strain of species used for the preclinical study is also listed. As most of the times combination of excipients is employed, the table also gives information on quantities in combination that were employed without causing any observable effect on the species under investigation.

After preparing a formulation best suited for its intended purpose, it is also necessary to characterize it for drug content, assay, and other relevant technique that would give first-hand information on its physical and chemical stability. Depending on the type of formulation being characterized, its testing parameter will change as they are varied in the type of excipient used, method of preparation, and its use. For a detailed list of the characterization parameters for the formulations or delivery systems mentioned, please refer to Table III.

PLANNING AND EXECUTION

From a pharmaceutical business perspective, one may venture that preclinical formulations do not carry the same perceived importance as do clinical formulations. However, they are critical because the future direction of the program is dependent on preclinical work which, in turn, may be dependent on the performance and appropriateness of the preclinical formulation. Also, like clinical studies, repeating preclinical studies can be extremely expensive. Therefore, good decision-making, careful planning, and right-first-time execution are essential for preclinical formulations. The preceding sections detail out the need for good decision-making in the choice and design of preclinical formulations. Wherever preclinical studies are being conducted in animals, the availability and management of animals drives the logistics of everything else that is needed in the studies. This is why one must carefully plan various steps and the timing leading up to and during the actual administration. Finally, on the day of the study, the formulator needs to help ensure that his or her activities are executed flawlessly as the study's success depends on it. Our approach has always been to make the formulator be an integral part of the study team and have him or her available to support the study with no competing commitments.

A pharmacokinetic study requires us to provide one or more formulations for administration, either prior to or on the day of administration. The animals are often in a fasted state, and most studies start early in the morning (to allow for 12 sampling hours), and hence timely delivery is critical. If the formulation supplies are to be provided in advance, we execute the delivery as we would a clinical supply, appropriately labeled and accompanied with clear directions regarding at-site preparation methods, if any, and administration methods. In our laboratories, the formulation is generally prepared on the day of the study. This is often to ensure minimum use of precious NCE in stability studies. Providing fresh formulation just before the start of the study eliminates the need for NCE consuming stability studies, although it does put a greater burden on the shoulders of the formulator. We now describe a typical example of how a preclinical pharmacokinetic study using an oral suspension may be supported in our laboratories.

A day in advance of the study, the pharmacokineticist submits a formal formulation request. This form indicates the doses, the volumes required, the species, the route of administration, etc. Due to good communication between the drug metabolism and pharmacokinetic (DMPK) and formulation departments, this information has generally been communicated well in advance and is not a surprise. Filling this form reinforces the request, avoids possibility of miscommunication, and allows for traceable documentation of the basis of the formulation. On the day prior to the day of dosing, we outline the area where the work will be done and lay out our needs like clean vessels, excipients, printed labels, etc. On the day of the study, the formulation scientist will be in the laboratory at least an hour in advance of the formulation delivery time, calibrate the balance, and weigh the NCE and excipients, and prepare the formulation in accordance with documented procedures established during development. Careful notes are taken in a laboratory notebook so that they can be referenced if the results of the study warrant a check. Once the formulation is prepared, the pH of formulation will be checked, and the formulation is transferred to suitably labeled vials. The label states the name of the project, the product, the strength or concentration, the date it was prepared, batch number, and instructions (e.g., "use immediately" or "shake well before use"). The formulation is then hand-delivered to the DMPK laboratories. Development data, where available, supports the in-use stability. Portion of the formulation is retained for simultaneous assay and characterization as may be necessary. All the procedures followed for preparing formulations in exploratory pharmacokinetic studies are applicable also to pharmacological efficacy studies in animals and in toxicity studies. When supporting a GLP preclinical study, in addition to all the above, well-established standard operating procedures (SOP) have to followed while preparing formulation to support toxicology studies (3). The formulation is appropriately characterized through quality checks by analytical department and is formally released for a study if the set specifications are met. Additionally, formal stability data on the formulation is required to support GLP long-term toxicity studies. The formulator needs to be trained with knowledge of The Organization for Economic Cooperation and Development (OECD) guidelines for the support of GLP toxicology studies (117). This ensures the generation and documentation of quality data that is aligned with any regulatory agency's GLP requirements.

CONCLUSION

Through the history of formulation development, the formulator has increasingly developed both breadth and depth. As each segment of a formulator's work has become increasingly complex, and moved from empirical to more scientific and methodical, more specialization has come into play. The preclinical formulator is an example of such a specialization. Preclinical formulations require a good understanding of fundamental science, exposure to

physicochemical characterization, and varied formulation techniques, an ability to make sound data and information-based choices and decisions, careful planning, flawless execution, and precise and detailed documentation. Much of the data and information on this "specialization" has unfortunately remained within the confines of major pharmaceutical companies' notebooks, as much of the tricks-of-the-trade provide a competitive edge. Lately though, a lot of good articles have made it into the public domain as have many good workshops and conferences been held on this area of specialization. We have attempted to summarize what we have found in the literature (although, we have consciously avoided summarizing other articles allowing the reader to reach them via the references listed in this paper) combined with our own practical learnings so as to provide the preclinical formulator a resource guide to access when developing and delivering preclinical formulations.

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Conflict of Interest Authors declare no conflict of interest.

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