Pharmaceutical Dosage Forms:

Parenteral Medications

Third Edition

Volume 1: Formulation and Packaging

Edited by
Sandeep Nema
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4 Preformulation

N. Murti Vemuri

INTRODUCTION

Parenteral medication refers to drugs administered by routes other than the oral, typically implying injectable medications. Injectable medications could be presented in various volumes (small volume and large volume), primary packaging (ampoules, vials, cartridges, bags) and specified routes (e.g., intravenous, intramuscular). Many of the preformulation and formulation principles applicable to injectable medications can often be extended to ophthalmic and nasal spray dosage forms as well.

Rational formulation development of parenteral medication should be based on the desired product profile, the physicochemical properties of the drug substance and its interaction with other formulation ingredients, primary packaging components under storage conditions defined by the product profile, as well as the pharmacokinetic properties of the drug substance. Preformulation research comprises pharmaceutical and analytical investigations in acquiring such knowledge base, and these investigations both precede and support formulation development.

On a drug development timescale, preformulation research enables data-driven decisions related to the drug substance and drug product such as salt form selection, polymorph selection, excipient selection, identification of suitable toxicology formulations, and, finally, selection of compositions for clinical and commercial formulations. Additionally, understanding the physical and chemical attributes of the drug substance can often help in troubleshooting formulation, stability, and processing issues that may arise.

Many good reviews and book chapters (1) have been written on the subject of preformulation and physicochemical characterization of drug substances. Although most articles focus on oral formulations, many of the principles carry over to development of parenteral medications. This chapter will attempt to focus more on aspects relevant to development of parenteral dosage forms. Much of the discussion will focus on small molecules and solutions dosage forms, but later sections will touch on specificities related to macromolecules and specialized dosage forms.

CHARACTERIZATION OF THE DRUG SUBSTANCE

Understanding the physicochemical properties of the drug substance is the first step (2) toward building quality into a product using rational formulation design. Drug substances are investigated at various levels of scrutiny to fully understand their behavior—at the molecular/material level, at the particulate level and also at a bulk property level. Table 1 shows a representation of this hierarchy of physicochemical properties. The intended dosage form often dictates where to place the greatest emphasis. For a solid dosage form, it is important to also fully understand the bulk properties, but for parenteral dosage forms, greater emphasis is on understanding the molecular and material properties of the active pharmaceutical ingredient (API).

Molecular Properties

Prior to initiation of preformulation studies, the molecular structure of the drug substance is identified and confirmed by appropriate spectroscopic (NMR, MS) evidence. The material is further identified by its characteristic IR and UV spectrum.

Physicochemical Constants

Two key physicochemical constants of importance are the partition coefficient and the ionization constant. The partition coefficient is an indication of the lipophilicity of a compound and is measured as a ratio of the equilibrium concentrations of the drug in an oily (e.g., octanol) and an aqueous (e.g., water) phase in contact with each other and held at a constant temperature. The

Table 1 Physicochemical Properties of Drug Compounds

Molecular properties *Properties defined by the molecular structure*	**Material properties** *Properties intrinsic to the material or particle*	**Bulk properties** *Properties related to bulk powders*
Molecular weight	Salt form	Powder flow
log *P*/log *D*, pK_a	Crystal form (XRPD)	Bulk density
Chemical stability	Crystal habit	Wettability
Melting point		Powder electrostatics
Solid-state stability		
Solubility		
Spectral characterization (UV, IR, NMR)	Particle size	
	Specific surface area	
	Hygroscopicity	

Abbreviation: XRPD, X-ray powder diffractometry.

logarithmic value of the ratio of these concentrations is often used and referred as log *P*, or partition coefficient. When an aqueous buffer solution (often pH 7.4) is used instead of water, the value is referred to as log *D*, or distribution coefficient. These coefficients, which are descriptions of the lipophilicity of a compound, are often correlated to the ability of a compound to cross biological membranes as well as their ability to dissolve in formulation vehicles.

The ionization constant (K_a), an intrinsic property of the molecule, describes the ionization behavior of a compound as a function of pH. The negative logarithm of K_a is often used and referred to as pK_a. The pK_a is equal to the pH value when the ratio of the ionized and unionized species is one. The pK_a is thus an important determinant in the pH dependence of ionization and hence solubility as well as salt formation ability of a molecule. These concepts will be further expanded elsewhere in this chapter. If a compound has multiple ionizable groups, each group has a corresponding pK_a value.

The molecular structure of the compound can be utilized for obtaining additional first estimate of properties such as dissociation constants and partition coefficients utilizing prediction software (e.g., from ACD/Labs, Simulations Plus, etc.). Such software packages can also provide a first estimate of the solubility and pH-solubility profiles. These data are especially useful during early development when compound supply is very short and there is a need to provide formulations for discovery pharmacology and early toxicology studies.

Solubility

Solubility is the concentration of drug in solution at equilibrium with excess solid. Typically, when the solid drug is brought in contact with a solvent, it dissolves into the solvent over a period of time and achieves equilibrium asymptotically. Aqueous solubility is of particular relevance to biological activity, bioavailability, and formulation strategy (3).

Solubility is experimentally measured by placing an excess solid in a test tube in contact with a particular solvent with mild agitation and determining the concentration of the drug in a supernatant solution over a period of time using appropriate analytical techniques such as UV spectrophotometry or high-performance liquid chromatography (HPLC). In determining equilibrium solubility, it is important to ascertain that (*i*) an asymptotic value has been achieved (constant over multiple time-points) and (*ii*) the identity of the solid in contact with the solvent is unchanged. The identity of the residual phase can be confirmed by analyzing the residue using techniques such as differential scanning calorimetry (DSC) or X-ray powder diffractometry (XRPD).

During preformulation studies, it is common to determine solubility of the drug compound in aqueous and nonaqueous vehicles used in pharmaceutical formulations. Aqueous systems include buffers, surfactant solutions, and complexant solutions. Nonaqueous

systems include cosolvents (e.g., ethanol, glycerol, polyethylene glycols) and oils (soyabean oil, glycofurol). A more detailed list of excipients is discussed later in this chapter.

pH-solubility profile. Many pharmaceutical compounds contain acidic or basic functional groups and hence show pH dependence in their aqueous solubility. Solubilities can vary significantly in accordance with the pK_a across acceptable pH range. Hence, adjusting pH to achieve requisite solubility can be an important tool in formulating injectable solutions.

The pH dependence of solubility of acids and bases is derived from the ionic equilibria occurring across the pK_a of a compound and is described by the Henderson-Hasselbalch equation (4).

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{A}^-]}{[\mathrm{HA}]} \quad (\text{for an acid}) \tag{1}$$

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{[\mathrm{B}]}{[\mathrm{BH}^+]} \quad (\text{for a base}) \tag{2}$$

Taking the example of a free base, the total solubility of at any given pH is the sum of the solubility of the unionized species (S_0) and the ionized species.

$$S = S_0 + [\mathrm{BH}^+] \tag{3}$$

Figure 1 shows a hypothetical pH-solubility profile for a weak base. At a high pH (pH >> pK_a), the solubility is practically independent of pH and is essentially S_0. As the pH approaches the pK_a, the fraction of ionized species and hence the total solubility increase and are described by

$$S = S_0\left(1 + \frac{[\mathrm{H}^+]}{K_a}\right) \tag{4}$$

The ionized species can associate with a charged counterion to form a salt. This linear increase in solubility ends abruptly when the solubility of the salt form is reached, and at this point the solubility is governed by the solubility product (K_{sp}) of the salt form. For example,

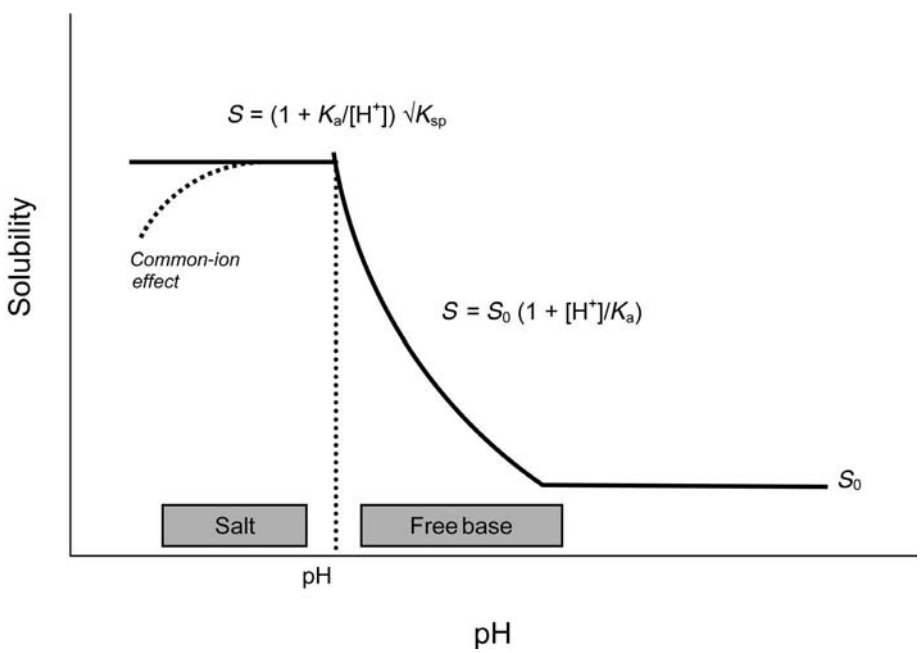


Figure 1 pH-solubility profile of a hypothetical weak base.

Table 2 Properties of Some Commonly Used Solvents

Solvent	Dielectric constant (ε)	log P	Surface tension (γ) (dynes/cm)
Water	81.0	−4.00	72.0
Glycerin	42.5	−2.60	64.9
Propylene glycol	36.7	−1.93	48.8
Ethanol	24.3	−0.31	22.2

assuming that the pH was being changed by titrating with hydrochloric acid, the solubility product is

$$K_{sp} = [BH^+][Cl^-] \tag{5}$$

and the total solubility at this pH_{max} would be

$$S = \left(1 + \frac{K_a}{[H^+]}\right)\sqrt{K_{sp}} \tag{6}$$

Rearranging the equation, the pH_{max} can be determined if the solubility product is known.

$$pH_{max} = pK_a + \log\frac{S_0}{\sqrt{K_{sp}}} \tag{7}$$

Common-ion effect or salting-out effect is also depicted in Figure 1, representing the pH-solubility profile of a weakly basic drug. From the pH of maximum solubility, as one moves toward lower pH values, there is an increase in the concentration of the counterion (e.g., $[Cl^-]$). Depending on the value of the solubility product (a function of the nature of the drug and the counterion), this increase may be compensated by a decrease in the concentration of the ionized drug molecule. This decrease occurs through a precipitation of the drug in its corresponding salt form. This phenomenon is known as "salting-out" or common-ion effect and can be an important consideration in selecting salt forms or buffer systems for formulations.

Solubility in cosolvent systems. Cosolvents such as ethanol, propylene glycol, polyethylene glycols, and glycerol are routinely used in formulating to a higher solubility when aqueous solubility alone is not sufficient to achieve required levels. In case of some drug compounds, the use of appropriate cosolvents can increase the solubility quite significantly. The mechanism behind the increased solubility is frequently related to modifying the polarity or dielectric constant of the solvent system. The principle of "like dissolves like" works—less polar molecules would be better dissolved in a less polar solvent system. Adding a cosolvent with a smaller dielectric constant to water will bring down the overall dielectric constant of the resultant solvent system and make it a better medium for dissolving a less polar or nonpolar molecule. Table 2 shows some physical parameters of common cosolvents (5).

Although cosolvents can be quite effective in achieving solubilization, it should be noted that as excipients these can have toxicological effects (e.g., hemolysis) and potential for local irritation depending on the concentrations used. Additionally, it is very important to consider the potential for the drug to precipitate upon dilution (6). This risk can be assessed both by calculating the degree of precipitation that could occur and by experimentally simulating the dilution that could occur and testing for precipitation potential (7).

Solubility in surfactant systems. Surfactants, a common class of excipients, are amphiphilic molecules (hydrophilic head group and hydrophobic tails), which strongly orient themselves at interfaces. In an aqueous system surfactant molecules would mainly be present at the water-air interface with a small but finite concentration in the bulk of the solution. Surfactants oriented at the water-air interface cause a reduction in the surface tension of water and thereby

improve wettability of drugs being exposed to such a system. With increasing concentration of surfactant in the system, the interface becomes crowded, and at a specific concentration, the surfactant molecules in the bulk orient themselves in micellar structures. Micelles consist of spherical structures with the hydrophobic (lipophilic) tails toward the core and hydrophilic heads forming the external surface. The concentration at which this occurs is called the critical micelle concentration (CMC). Above the CMC, aqueous surfactant systems would contain micellar structures in the bulk.

Lipophilic drugs can be incorporated into the core of micelles, thereby increasing the total solubility of a drug into aqueous systems. The lipophilic cores of micelles present a different environment to the drug molecule providing, in some instances, a stabilizing effect against chemical degradation. Surfactants will preferentially orient toward the surface of nuclei during a precipitation phenomenon and can prevent precipitation occurring due to dilution effects. Thus, surfactants can be a very useful tool in formulating aqueous injectable solutions and suspensions.

Examples of surfactants commonly used in injectable formulations include polyoxyethylene sorbitan monoesters (Tweens), polyoxyethylene-polyoxypropylene copolymers (Pluronics), sodium lauryl sulfate, and lecithins.

Solubility in complexant systems. A complex is an entity formed when two molecules, such as a drug and a solubilizing ligand, are held together by weak, noncovalent forces (dipole-dipole, hydrophobic, or hydrogen bond interactions). Cyclodextrins are a class of such solubilizing ligands that have found a significant application to pharmaceutical compounds. α-, β-, and γ- Cyclodextrins are cyclic oligomers of glucose containing six, seven, or eight glucose residues. Cyclodextrins have gained popularity from a pharmaceutical standpoint because of the ability of these materials to interact with poorly water-soluble drugs and drug candidates resulting in an increase in their apparent water solubility. The mechanism for this solubilization is rooted in the ability of cyclodextrin to form noncovalent dynamic inclusion complexes in solution. As a result of their structure, cyclodextrins present a hydrophilic exterior but a core that is more lipophilic and hence provides a microenvironment for lipophilic drug molecules to engage via hydrophobic interactions. In certain cases, the modified microenvironment of the cyclodextrin core results in improved chemical stability similar to micellar systems. The ability of the cyclodextrin to solubilize a drug compound depends on steric factors (size of the cavity) and thermodynamic factors (decrease in free energy of the system). Additionally, the solubility of the cyclodextrin in water is another key determinant. β-Cyclodextrin has relatively low water solubility (~18.5 mg/mL), but chemical modifications of the basic β-cyclodextrin have imparted improved solubility and lower toxicity. Two of the modified β-cyclodextrins that have gained greater acceptance are hydroxypropyl-β-cyclodextrin (HP-β-CD) and sulfobutylether-β-cyclodextrin (SBE-β-CD). These have water solubilities of about 600 mg/mL and 500 mg/mL, respectively. Both of these modified cyclodextrins have been used in developing injectable formulations that are now FDA-approved products.

During preformulation studies, it is common to assess the solubility of a poorly soluble drug candidate in such cyclodextrins. If solubilization via cyclodextrin complexation is identified as a potential formulation approach, then it is also important to fully characterize the interactions in terms of stoichiometry of the complex as well as the equilibrium constant for the complexation. A number of excellent reviews cover the theoretical and experimental considerations for such determinations in detail (8–10).

Stability and Drug Degradation

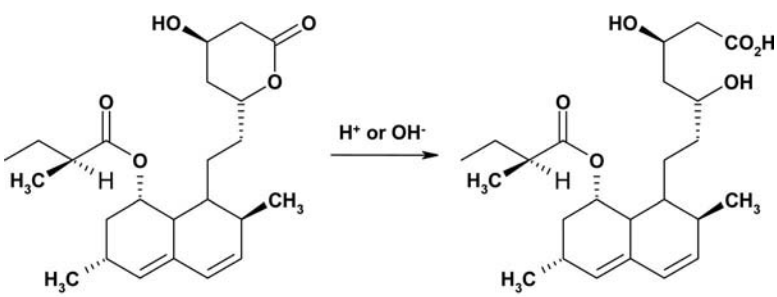
In addition to solubility, stability of the active drug compound is a key determinant in the viability of parenteral drug product. First, it is essential for a drug product to maintain potency relative to label claim over the shelf life to deliver an accurate dose. Second, degradation in the drug product can result in changes in appearance (color, precipitation) or bioavailability. Finally, degradation of the active compound can result in degradation products that may have toxicity that is more significant than that of the active drug substance. Depending on the daily

dose of the active and levels of such degradation products anticipated in a drug product, they may be subject to additional toxicological qualifications as described in ICH Guidance Q3B (R2) (11). When impurities or degradation products are identified as potentially genotoxic, they have to be controlled to very low levels if not completely avoided. This process is detailed in the EMEA Guidance on Limits for Genotoxic Impurities (12). Thus, it is essential to understand the stability and degradation of the active ingredient as a bulk drug substance and in formulation. Understanding the degradation pathways, kinetics, and mechanisms leads to development of a stable drug product (13,14).

During preformulation studies, the goal is to understand the modes of instability of a drug compound, kinetics of degradation, and factors (including formulation factors) influencing the kinetics of such degradation (15). One of the first steps is to develop a stability-indicating method that is capable of resolving and quantifying impurities and degradation products resulting from the drug compound. Typically, HPLC with UV detection is used in preformulation studies, but techniques such as LC-MS and NMR spectroscopy could often aid in the identification of degradation products. HPLC methods are developed to effectively resolve degradation products resulting from forced degradation studies (highly stressed condition of temperature, humidity, or pH).

Modes of degradation. Chemical degradation of small-molecule drugs can occur because of various chemical processes. However, a majority of these fall into three types of reactions.

Hydrolysis This is a very common pathway for drug degradation (16) and is essentially the cleavage of a molecule under the effect of water. Since water, either as a solvent or in the form of moisture in the air, is ubiquitous, the potential for this degradation pathway exists for most drugs. This is of particular relevance to parenteral products, which are mostly formulated in aqueous systems. Chemical bonds that commonly undergo hydrolytic degradation include lactam, ester, amide, and imide bonds. Aspirin is the most common example of a drug undergoing hydrolytic degradation. Lovastatin is a prodrug that undergoes activation through hydrolysis by carboxyesterases in vivo. In vitro it undergoes hydrolysis under acidic and basic conditions by cleavage of the lactone.



lovastatin
(hypolipidemic agent)

Hydrolytic reactions can be significantly influenced by the composition of the medium—pH, buffer concentration, ionic strength, etc. The relationship of the rate of reaction (expressed as rate constant k_{obs}) with pH is quite informative both for understanding the mechanisms involved as well as a guide for formulation. When the reaction is catalyzed by the hydronium (H^+) ion, it results in a slope of negative one on a log k_{obs} versus pH profile, and similarly, when the reaction is catalyzed by the hydroxide ion (OH^-), a slope of one is observed. If no other catalyses are involved, then these two lines meet, forming a V-shaped profile. The pH at which they meet represents pH of maximum stability and is important to know during selection of formulation pH. The shape of curves can be more complicated (U shaped, additional inflections, etc.) depending on the number of ionic species involved (15,17).

In addition to the pH of the medium, concentration of the buffer itself can play a catalytic role in hydrolysis. This can be studied by studying the reaction rate as a function of buffer type and concentration while holding the pH constant. Ester hydrolysis of an experimental compound GW280430A was shown to be catalyzed by citrate, malate, and tartrate buffers but not by a glycine buffer (18). This phenomenon is termed as general acid/base catalysis or buffer catalysis and can often be the cause of deviation from a slope of −1 or +1 described in specific acid/base catalysis in the previous paragraph. Additionally, reactions can also be affected by ionic strength, which can be studied by holding the pH and buffer concentration constant and studying the reaction rate as a function of concentration of added ions (e.g., NaCl). Typically, this is not a big effect in pharmaceutical systems.

In summary, hydrolysis is a key degradation pathway for many drug compounds. pH-stability profiles can vary from a simple V shape to more complex profiles depending on the number of ionization states and the different reactivities they present. While some of the pathways can be predicted on the basis of the structure, evaluation of the pH-stability profile and effect of buffer catalysis can be very important in designing the formulation strategy.

Oxidation Oxidation is another common mode of drug degradation. Oxidation can be broadly defined as a loss of electrons in a system; alternately, it could be considered as an increase in oxygen or a decrease in hydrogen atoms. The reaction occurs in concert with reduction of the other reactant, thus forming a redox reaction. If molecular oxygen is involved in the reaction, this is termed as "auto-oxidation." Trace metals and light can catalyze oxidation reactions by initiating free radical chain reactions. Once formed, the radical can be propagated until a termination reaction or a suitable chemical inhibitor intervenes. These reactions can happen in aqueous and nonaqueous media.

Excipients used in formulation can be a source of trace metals and also peroxides, which can have significant effect on oxidative drug degradation. Table 3 shows levels of hydroperoxides measured in some commonly used pharmaceutical excipients (19).

To control oxidation reactions, antioxidants are often included in a formulation. Antioxidants used in a formulation could affect different stages of an oxidation reaction. True antioxidants (e.g., butylated hydroxy toluene, α-tocopherol) react with free radicals, resulting in termination of the chain reaction. Reducing agents (e.g., ascorbic acid) get preferentially oxidized and hence reduce the level of oxygen or the oxidant in the formulation. Chelating agents such as EDTA sequester trace metals which can catalyze oxidation and thereby function as antioxidant synergistic agents. Depending on the reaction involved, a combination of such agents may help control the oxidative degradation (20). Also, during manufacturing and in the primary package, an inert atmosphere generated by nitrogen blanketing can help control oxidative degradation.

Photolysis Photolysis, also referred to as photodegradation, occurs as a result of absorption of light (or radiation energy) (21). When the absorbed energy dissipates through a chemical change in the molecule, photolysis occurs. The changes may result in a color change, precipitate formation or may not be visually detectable. However, there is always loss of potency that is accompanied. Toxicity of the decomposition products is also of concern,

Table 3 Levels of HPO in Some Commonly Used Excipients

Excipient	Number of batches tested	Average HPO (nmol/gm)	Range of HPO (nmol/gm)
Polyvinylpyrrolidone	5	7,300	3,600–11,000
Polyethylene glycol 400	4	2,200	1,000–3,300
Polysorbate 80	8	1,500	180–4,600
Poloxamer[a]	7	30	10–50
Mannitol	5	<10	<10
Sucrose	5	<10	<10–20

[a]Different grades (188, 338, and 407) and batches tested.
Abbreviation: HPO, hydroperoxides.

especially when such products can form by the action of sunlight on the skin or eyes after administration (phototoxicity) (22).

Photodegradation depends on wavelength of the incident light as well as intensity. Primary photochemical reactions usually occur at wavelengths where the drug absorbs light, that is, in regions where the UV/VIS absorption spectrum of the drug overlaps with the spectrum of incident radiation. In some instances it is possible that the energy absorbed by a nondrug molecule (photosensitizer) in the formulation is transferred to the drug molecule, which eventually degrades. Examples of some common drugs that undergo photolytic degradation include methotrexate, furosemide, and tetracyclines. For many drug substances, the kinetics of photodegradation varies significantly with the ionization state of the molecule. Examples would include ciprofloxacin, midazolam, mefloquine, and ameloride (23).

Once a photoinstability is identified, it can be addressed during formulation development through different means. A protective market pack is one of the simplest solutions. Control of pH, ionic strength, trace metals, or even use of complexants (24) can be formulation approaches to also address such instability.

In addition to these major modes of degradation, many other routes are involved in drug degradation such as decarboxylation, racemization/epimerization, acylation, etc. Understanding the causes of drug instability allows for a rational design of a formulation.

Preformulation stability studies. Typically, the drug substance is studied in solid as well as solution states. Stability studies might involve storing the samples under stressed conditions of temperature and humidity such as 40°C/75% RH and 50°C. If the drug is fairly stable, conditions such as 80°C/75% RH and 80°C may be employed to get a first view of drug instability in a reasonable amount of time. These studies are conducted over a short duration such as four to six weeks.

Additionally, the solid drug and an aqueous solution of the drug are exposed to a representative duration and intensity of light in appropriate photostability chambers [as per ICH Q1B (25)]. These studies may be able to indicate not only potential need for protecting the drug product from light but also the need for conducting other stability studies under light-protected conditions. Failure to know this early can produce confounding results.

pH-stability profiles are determined by preparing aqueous solutions of the drug at various pH values ranging from 2 to 12 and studying the kinetics of degradation (loss of active/growth of degradation products) at an appropriate elevated temperature. The solutions are sampled at regular intervals and analyzed using a stability-indicating method. The time course of degradation at a particular pH can typically be expressed as the first-order rate constant k_{obs} (k observed). A log k_{obs} versus pH plot is referred to as the pH-rate profile and can be quite revealing of the mechanisms involved in drug degradation. The pH of maximum stability would be targeted as the pH for the formulation as long as it agrees with the required solubility and local tolerability at that pH.

Form Selection

The solid form of the drug compound can have a significant effect on parenteral drug product processing. During late discovery or early development stages, the solid form of the drug compound needs to be defined and fixed to develop formulations and processes consistent with the expected physical and chemical properties of the API. The solid form is typically described by the salt form used and the crystal polymorph of the chosen salt.

Salt Form

Many drugs are either weak acids or weak bases and can consequently form a range of salts by reacting with various bases and acids, respectively. Salt formation may be employed to alter the physicochemical, biopharmaceutical, and processing properties of a drug substance without modifying the pharmacologically relevant moiety (26).

To form stable salts, the pK_a of the basic center should be greater ($\Delta pK_a \geq 2$) than the pK_a of the conjugate acid to be utilized. Thus, for a basic drug, pK_a of the basic center will determine what salts are feasible.

In the case of parenteral medications, increased solubility is often desired from chosen salts. In general, utilizing counterions with greater acidity, utilizing more hydrophilic counterions (hydroxy acids), and lowering the melting point of the resultant salt (decreased crystal lattice energy) can result in increased solubility. Agharkar et al. (27) demonstrated an increased solubility of an experimental antimalarial drug as a result of decreased crystal lattice energy due to salt formation.

In the case of solution formulations, it is not essential that salt formation is only employed for obtaining a suitable solid form. Salts can be formed in situ in solutions by using the appropriate acid or base to adjust pH of the formulation (28). Sometimes the high aqueous solubility achieved prevents a salt from being easily isolated but can still be utilized as an effective solubilization approach, as previously discussed in the context of pH-solubility profiles.

Polymorph Selection

Polymorphism is defined as the ability of a substance (of constant chemical composition) to exist in two or more crystalline phases that differ in crystal packing arrangement and/or conformation of the molecules in the crystal lattice. The different crystalline forms are then termed as polymorphs.

Crystals are made up of repeating blocks called unit cells. Different polymorphs have distinct unit cells. Polymorphs can differ in various physical, physicochemical, and physicomechanical properties. Differences such as melting point, enthalpy of melt, true density, and powder X-ray diffraction patterns help characterize and differentiate between polymorphs. One can screen for polymorphs by crystallizing a drug from different systems of solvents, evaporation and cooling profiles, and then examining crystals obtained. However, it is not easy to search exhaustively for all possible crystal forms, and often new forms are discovered during development. To reduce the risk, many automated crystallization systems have been developed, which help examine a larger experimental space.

Polymorphism is commonly of concern in the context of solid dosage form bioavailability and processing (29). However, polymorphs also differ in properties that impact a parenteral drug product formulation of which solubility, dissolution rate, and hygroscopicity are of most relevance. Polymorphs differ in their free energy as a result of their packing, and this manifests itself as differences in solubility. The most stable polymorphic form has the lowest solubility. If a metastable polymorph is used in a solution or suspension formulation, there will be a risk of growing crystals of the stable form over a period of time. Solvent maturation studies and temperature cycling of prototype formulations can help identify such problems early.

When a solvent molecule incorporates itself into a crystal lattice associated with a drug compound, it is said to form a solvate. When this solvent is water, it is termed as a hydrate. A hydrate form of the drug is more stable than an anhydrous form and will exhibit lower solubility in an aqueous system. Thus, it is also important to understand and characterize solvate and hydrate forms of the drug compound.

Characterization of Material Properties

Appearance and Microscopy

The solid form of a drug substance is characterized by its appearance in terms of color and subjective description. Additionally, examination under a microscope reveals further details such as crystal morphology and habit.

Crystallinity

Crystalline material can be identified by polarized light optical microscopy where the sample displays birefringence. Crystallinity is also commonly examined by XRPD. An X-ray diffraction pattern is generated because of constructive and destructive interference of X rays reflected off the crystal planes of a powder sample as the angle of incidence is varied. This is described by the Bragg equation.

$$n\lambda = 2d \sin\theta \tag{8}$$

where θ is the incident angle, λ is the wavelength of the X radiation, d is the distance between the crystal planes, and n is an integer representing the order of reflection.

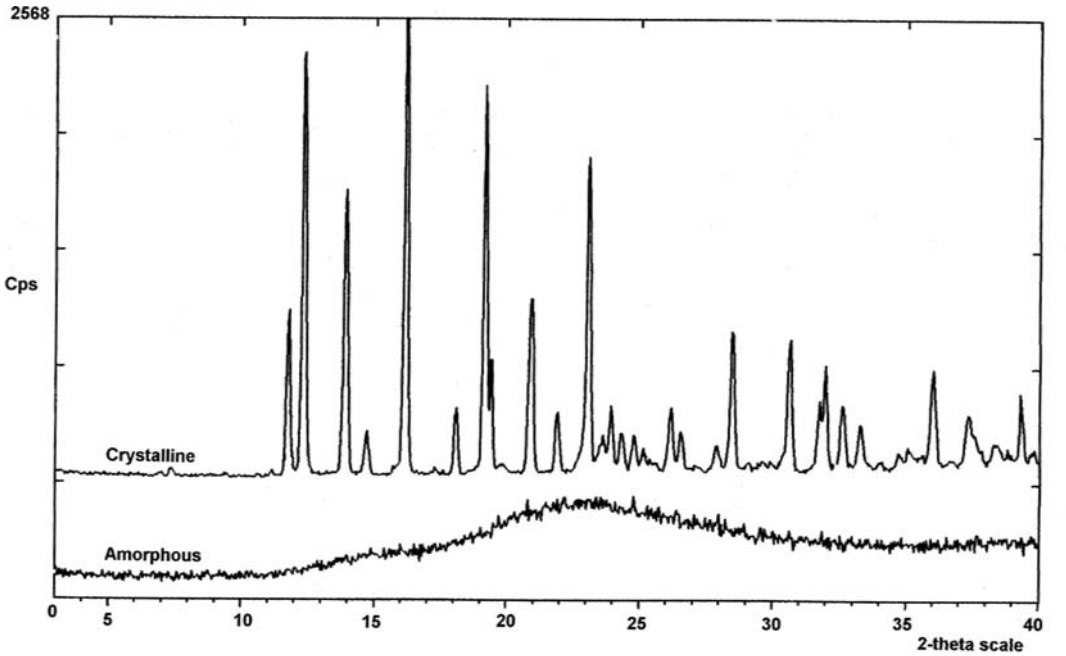


Figure 2 X-ray powder diffraction patterns showing amorphous and crystalline states of an experimental drug compound.

Crystalline forms are characterized by sharp characteristic peaks, while an amorphous material displays a broad halo (Fig. 2) (30). XRPD can be used to distinguish between different polymorphs, solvates, and hydrates. Further, this technique can also be used to quantify mixtures of polymorphs and degree of crystallinity of a crystal form.

Thermal Properties

DSC measures the difference in the amount of heat required to raise the temperature of a sample and a reference as a function of a change in temperature. A typical output shows heat flow into (endothermic event) or out of (exothermic event) the sample as a function of temperature. Melting of a crystalline material is observed as an endothermic event characterized by an onset temperature (melting point) and heat of fusion measured as the area under the endothermic curve. At the glass transition temperature, amorphous materials undergo a transition from a glassy rigid state to a rubbery state of greater mobility (a higher heat capacity), and this is observed on the DSC as a baseline shift characterized by temperature (T_g) and change in heat capacity (ΔC_p). The glass transition is sometimes followed by a small endotherm of enthalpic relaxation related to time-dependent relaxation of this phase. Figure 3 shows the DSC thermogram of an experimental drug compound displaying these transitions along with an overlay of corresponding changes to the X-ray diffraction patterns as observed by variable-temperature XRPD (31).

Modulated DSC (mDSC) is a related technique where an oscillation of temperature is introduced on top of a linear heating rate. This allows deconvolution of the output into reversing (thermodynamic) and nonreversing (kinetic) components, allowing a further understanding of the transitions measured. This can be of particular utility in studying amorphous materials (29).

Thermogravimetric analysis (TGA) measures the weight of the sample as a function of increasing temperature. Loss of water, solvents, or volatile decomposition products can be observed as a weight loss at characteristic temperatures. This analysis is a key technique in characterizing solvates and hydrates. The technique is sometimes further coupled with an IR spectrometer or a mass spectrometer to characterize the evolved volatile components that come off during heating of the sample.

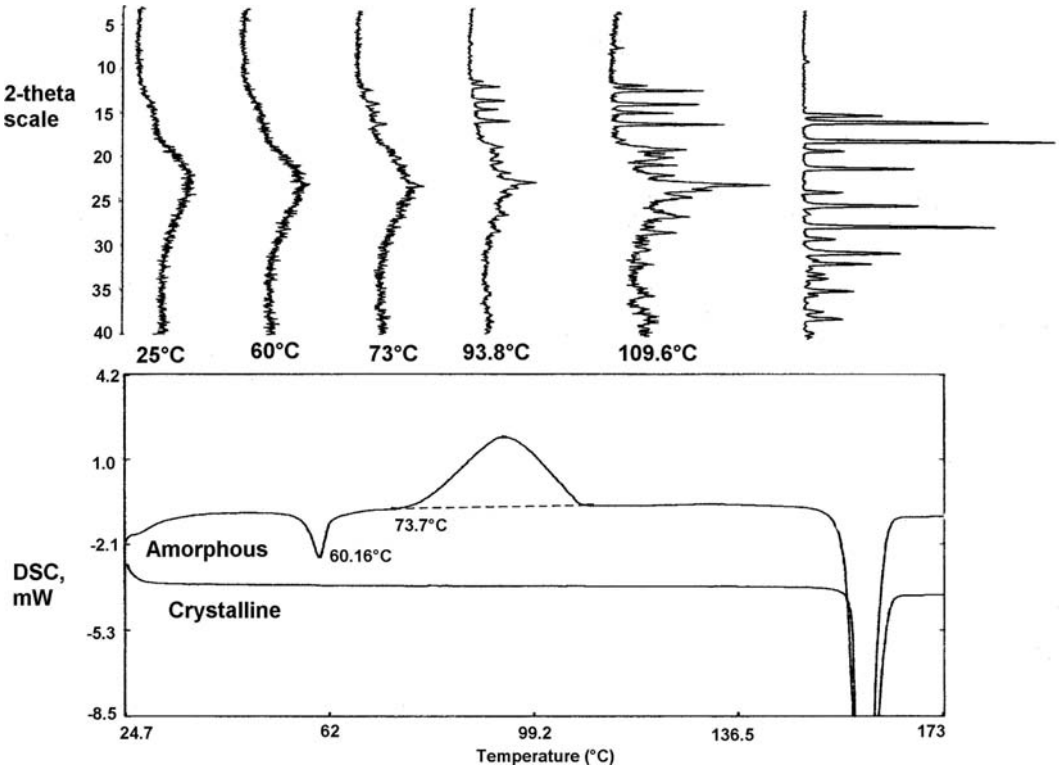


Figure 3 DSC curves of crystalline and amorphous phases of an experimental drug compound overlaid with XRD patterns of the amorphous phase obtained at temperatures corresponding to thermal events in the DSC curve. *Abbreviations*: DSC, differential scanning calorimetry; XRD, X-ray diffraction.

Vapor (moisture) Sorption Analysis

The weight of the sample is monitored as it is exposed to different relative humidities for a period of time approaching equilibrium. The output is a moisture sorption profile, which depicts the sample weight as a function of relative humidity. When a material picks up enough water that causes a change in its physical properties, the material is considered hygroscopic. Crystalline materials typically *adsorb* small amounts of water on the surface unless they pick up water molecules into the crystal lattice to form hydrates. Hydrates are characterized by picking up stoichiometric amounts of water and are physically stable over a range of %RH. Deliquescence occurs when the material adsorbs enough water to dissolve into it thereby turning liquid. This can sometimes happen with salts of hydrophilic molecules and is characterized by a sharp increase in moisture uptake at humidity values greater than a threshold %RH.

Amorphous materials *absorb* water and other solvents into the bulk. The absorbed solvent acts as a plasticizer and reduces the apparent glass transition temperature. When the apparent glass transition temperature drops below storage temperature, the material goes into a mobile rubbery state from which collapse of the structure (liquefaction) with possible recrystallization can occur. This relationship of glass transition temperature as a function of absorbed water is critical to understand when developing a lyophilization process.

INTERACTION BETWEEN THE DRUG SUBSTANCE AND FORMULATION COMPONENTS

Formulation Components

In formulating a parenteral drug product, a number of excipients are employed, and these often form the bulk of a drug product. These excipients are included to dissolve the drug substance, increase the chemical or physical stability of the drug product, give the product

Table 4 Excipients Used in Parenteral Formulations

Solvents and cosolvents
- Glycerin
- Propylene glycol
- Ethanol
- Polyethylene glycol (300, 400)
- *N,N*-dimethylacetamide
- Soyabean oil
- Corn oil
- Ethyl oleate
- Glycofurol

Surfactants (solubilizers, emulsifiers, and suspending agents)
- Polysorbate 80 (Tween 80)
- Polysorbate 20 (Tween 20)
- Polyoxyethylene-polyoxypropylene copolymers (poloxamers)
- Cremophor EL
- Lecithin

Complexants
- Hydroxypropyl-β-cyclodextrin
- Sulfobutylether-β-cyclodextrin (Captisol®)

Buffers
- Citrate
- Phosphate
- Tartrate
- Tromethamine (TRIS)

Chelating agents
- Disodium ethylenediaminetetraacetic acid

Antioxidants
- Ascorbic acid
- Butylated hydroxy anisole
- Butylated hydroxyl toluene
- Sodium bisulfite
- Propyl gallate
- α-Tocopherol

Preservatives
- Benzalkonium chloride
- Benzethonium chloride
- Benzyl alcohol
- Chlorbutanol
- Paraben (methyl, propyl)
- Thimerosal

Tonicity adjusters, bulking agents, lyoprotectants
- Sodium chloride
- Mannitol
- Glycine
- Sucrose
- Trehalose
- Dextran
- Povidone

microbiological protection, or control other product attributes. Since inclusion of new additives could require extensive pharmacological and toxicological evaluation, it is common for formulators to depend on materials already used in marketed parenteral products. Table 4 shows a representation of the classes of excipients that might be used in parenteral formulations and some examples of each of these categories. There is more discussion within this book on the functions and levels of these excipients. Additionally, the reader can refer to some excellent reviews that have been published on this topic (31,32). The FDA also maintains a listing of inactive ingredients used in approved products (33).

Designing Excipient Compatibility Studies

Excipients are often referred to as inactive or inert ingredients to distinguish them from the APIs. However, the lack of pharmacological activity does not necessarily result in a lack of chemical reactivity. Excipients can have significant expected and unexpected effects on the physical and chemical stabilities of a drug product. This is first assessed through well-designed excipient compatibility studies conducted at the preformulation stage (34).

Traditionally, thermal methods such as DSC have been employed as a first screen in determining incompatibilities (35). In these studies, the drug, excipient, and drug-excipient mixture are subjected to a temperature program. If the thermogram of the mixture is not representative (temperature and enthalpy) of the combination of the two single components, then an incompatibility could be suspected. Modifications such as a stepwise isothermal high-sensitivity DSC study have also been tried (36). However, DSC techniques have proved to be of limited predictability.

Isothermal heat conduction calorimetry is a technique that measures heat evolved or absorbed by a sample (relative to a suitable reference) with great sensitivity. Hence, even slow reactions occurring under isothermal (25°C, 45°C/75% RH) can be detected because of the

Table 5 A Plackett–Burman Design

	Variable											Response
Trial	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	Y
1	+	+	−	+	+	+	−	−	−	+	−	
2	+	−	+	+	+	−	−	−	+	−	+	
3	−	+	+	+	−	−	−	+	−	+	+	
4	+	+	+	−	−	−	+	−	+	+	−	
5	+	+	−	−	−	+	−	+	+	−	+	
6	+	−	−	−	+	−	+	+	−	+	+	
7	−	−	−	+	−	+	+	−	+	+	+	
8	−	−	+	−	+	+	−	+	+	+	−	
9	−	+	−	+	+	−	+	+	+	−	−	
10	+	−	+	+	−	+	+	+	−	−	−	
11	−	+	+	−	+	+	+	−	−	−	+	
12	−	−	−	−	−	−	−	−	−	−	−	

sensitivity of the technique (37). This technique has been used to compare the heat signal from a drug-excipient mixture with the sum of the curves generated by the individual components under the same conditions. The magnitude of this interaction curve (difference curve) is an indicator of the extent of the incompatibility (38). However, this technique generally suffers from the fact that it is nonspecific and it is important to carefully design appropriate control experiments to make sure that the recorded heat pertains to a specific chemical incompatibility.

Given some of the challenges described above, the conventional method of chemical analysis of mixtures stored under accelerated storage conditions is still the most commonly employed method. The prerequisite for this methodology is having a stability-indicating method, and most commonly, this is an HPLC method. Since in a parenteral product, the drug and excipients are in very close contact (at a molecular level in the case of solution products) with each other, the stabilizing or destabilizing effect of an excipient is best studied in the presence of all formulation components (prototype formulations) including the targeted primary packaging when possible. High and low levels of each excipient or formulation factor are identified for testing on the basis of conventional levels used in experience or levels approved for use by regulatory authorities.

Different experimental designs can be used for obtaining the required information from a limited number of experimental runs. In such studies excipients constitute factors (at two levels—high and low) in a factorial design of experiments. For such studies screening design is employed at first. A commonly used screening design is a fractional factorial design called a Plackett–Burman design. Table 5 represents a possible design for studying 11 factors by performing 12 trials. This design was employed for a parenteral preformulation study for Naproxen as described by Peswani and Lalla (39). In this study they looked at effects of five excipients, pH, buffer type, autoclaving, and nitrogen blanketing by conducting 12 trials. Although these designs are quite efficient in terms of number of trials, it should be noted that these designs are not capable of identifying interaction terms (e.g., if two factors interact to produce an effect). If such confounding is suspected and needs to be resolved, a full factorial design study could be conducted on a smaller number of identified factors. The reader can get details of the advantages and disadvantages of different experimental designs from other reviews of this specific topic (40).

INTERACTION OF THE DRUG WITH PACKAGING COMPONENTS AND MANUFACTURING SURFACES

Parenteral drug products are in close contact with the primary package of the drug product; so it is useful to carefully consider primary package in the same way other formulation ingredients are evaluated. These packaging materials would include glass vials (or ampoules),

rubber stoppers, infusion bags, etc. Glass vials are most commonly type I (borosilicate glass), but that too can undergo different surface treatments at the manufacturer. Rubber stoppers (commonly butyl or other synthetic rubber and rarely natural rubber because of its sensitizing potential) and bag materials can be quite complex in composition. The formulation scientist works closely with the rubber manufacturer as with the glass manufacturer to choose the appropriate rubber formulation having consistent specifications and characteristics to maintain product stability. It is important during preformulation studies to include an evaluation of likely primary packaging materials to assess potential issues such as adsorption and incompatibilities. Also important to consider are other likely surfaces to be encountered during manufacturing steps, for example, stainless steel, glass, tubing, and filters.

Adsorption

Adsorption occurs when a molecule is attached to another solid surface, most commonly because of Van der Waals forces, hydrogen bonding, or electrostatic interactions. This can often occur with low-solubility hydrophobic compounds as they may prefer another surface as opposed to being in water. When a covalent bond is involved, the adsorption is chemisorption, but this is not commonly observed in the systems being discussed here.

To evaluate adsorption, the formulation (at the most dilute concentration likely) is exposed to the surface and then assayed for loss of drug concentration. For filters and tubing, this might involve passing through the tubing and filters for a fixed duration of time that will exceed the likely duration of a manufacturing run. For stoppers, it might be done by adding a fixed number of stoppers to flasks containing the formulation and storing for a fixed period of time before assaying the concentration. During development of an injectable formulation of Abbott-72517, Gupta et al. observed a 6% of loss of drug (250 mL recirculated for four hours) using a Pall Nylon 66® filter but no loss with a Millipore Durapore disk membrane (41). If adsorption to potential surfaces is identified early on, then it can be used to select appropriate materials for packaging and manufacturing processes.

Compatibility

In addition to adsorption, the degradation of the drug molecule can also be effected by packaging material or manufacturing surfaces. Thus, when feasible, it is useful to conduct excipient compatibility studies using preferred container closure systems. An early readout on any potential incompatibility can lead to an early assessment of alternatives and prevent the loss of time during development. For instance, rubber stoppers can leach out trace quantities of zinc into the formulation and effect oxidation of the drug. If a drug is particularly prone to oxidation, a steel surface may aggravate the issue and a glass-lined tank may be an appropriate measure. Protein drugs could be especially sensitive to silicone that is used on rubber stoppers. A nonsiliconized rubber with a bonded coating may be the answer to the issue.

SPECIALIZED FORMULATIONS

Suspensions and Nanosuspensions

Sterile injectable suspensions comprise of the active compound dispersed in a liquid vehicle either as a ready-to-use formulation or as a dry powder for reconstitution. Such formulations may be engaged either when the drug has solubility that is too low for a solution formulation or for prolonging the release of the drug through depot formulations. Aristocort® is a suspension of triamcinolone diacetate and may be administered by the intramuscular, intra-articular, or intrasynovial routes depending on the situation (42). NPH insulin is a suspension of crystalline zinc insulin combined with the positively charged polypeptide protamine. When injected subcutaneously, it has an intermediate duration of action. Depo-Medrol® is an anti-inflammatory glucocorticoid for intramuscular, intra-articular, soft-tissue, or intralesional injection. One of the challenges of formulating such products involves an evaluation of suspension physical stability with regard to resuspendability and caking.

Another area of specific concern for suspensions is syringeability (drawing a uniform dose) and injectability (pressure applied to expel product through a needle of specified gauge) of the product. The flow properties of the suspension can be characterized using techniques such as rheometry. This technique characterizes the flow of a fluid in response to a range of

applied stresses, resultant strains, and temperatures. Many suspensions and emulsions do not show a linear relationship between applied stress and strain (non-Newtonian behavior) and hence cannot be characterized by a single value for viscosity. A full discussion of this topic is out of the scope of this chapter and is well captured in many reviews on this topic.

For suspension formulations, the solid-state properties are quite relevant. Particle size of the dispersed phase can have a significant impact on the physical stability and syringeability of a suspension. Particle size distributions in suspensions can change over time because of Ostwald ripening—a solution-mediated phenomenon during which larger particles grow at the expense of smaller particles dissolving. An appropriately selected medium and surfactant can minimize the impact of this phenomenon. During screening, subjecting prototype samples to temperature cycling can accelerate the event and help select systems that are the most stabilizing. Crystal growth can also occur because of a more stable polymorph precipitating or a salt being formed. A change in crystal habit can result in significant effects on syringeability and injectability. Hence, there is a greater emphasis to fully understand the solid properties of the drug being formulated as a suspension as opposed to a solution product.

Lately, there has been a growing interest in formulating poorly soluble drugs as nanoparticulate suspensions (43). For compounds that exhibit poor solubility in aqueous and oily vehicles, nanosuspensions could be a preferred formulation option resulting in improved bioavailability. Nanoparticles also form an interesting platform for attaching targeting moieties. Nanoparticles are produced by "top-down" (media milling) techniques (44) or by "bottoms-up" (controlled crystallization) approaches (45). More recently, there have been reports of generating engineered nanoparticles by printing techniques (46).

Well-formulated nanosuspensions are typically nonsettling and hence circumvent some of the concerns mentioned previously with conventional suspension formulations. In such formulations the natural tendency of these small particles to aggregate is overcome by a careful selection of stabilizers, which could include a mix of surfactants and polymers. Compatibility of the drug with a range of possible surfactants and polymers needs to be assessed in parallel to selecting the best options for stabilization. As in conventional suspensions, Ostwald ripening and crystal growth is a concern, and gaining a good understanding of the solid-state properties of the drug is very relevant. Prototype nanosuspensions can be stressed by temperature cycling and freeze-thaw studies to establish their physical stability. It is also useful to assess the physical and chemical stability of the formulated drug to autoclaving conditions to define the strategy for sterilization.

Emulsions

Injectable emulsions have been most commonly used for long-term parenteral nutrition (Intralipid®, Lipofundin®). However, emulsions can also be good carriers of drug substances with good lipid solubility (high log *P*) and poor aqueous solubility (47). Propofol (Diprivan®) and diazepam (Diazemul®) are examples of drugs formulated as emulsions (33), and there are reports on studies conducted with Taxol emulsions (48). With the increased interest in injectable lipid emulsions, there is also a greater awareness of safety issues surrounding such delivery (49).

Typical emulsion formulations consist of oils (long- and medium-chain triglycerides or high-quality food grade oils), emulsifiers (e.g., lecithins, poloxamers, Tweens, and Spans) and an aqueous phase containing appropriate additives to control pH, tonicity, etc. Antioxidants such as α-tocopherol could be included in the oil phase to prevent oxidation of the oils. The emulsions are typically prepared by dissolving the appropriate ingredients in the oil phase and water phase and then homogenizing (e.g., Microfluidizer®, Silverson® homogenizer) the two to obtain the emulsion.

Some attributes to be studied in the specific context of emulsion formulations include assessment of particle (droplet) size and surface charge. Droplet surface charge is measured in terms of the zeta potential. Essentially, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. The zeta potential is determined using instruments that measure the elecrophoretic mobility of the particles. The surface change on droplets stabilizes emulsions because of electrostatic repulsion, which prevents coalescence of droplets. A zeta potential of ±30 mV or higher can

help stabilize a colloidal system. Measurement of zeta potential is equally useful while formulating suspensions and nanosuspensions.

SPECIFICITIES RELATED TO BIOLOGICS

Biotherapeutic molecules could range from small oligonucleotides or peptides synthesized using techniques such as solid-phase synthesis to proteins (including interferons, soluble receptors, antibodies, etc.) with tertiary and quaternary structures, which are often produced via genetic engineering technologies. Small oligonucleotides and peptides can often be formulated and analyzed by techniques similar to small molecules. More specialized analytical techniques and formulation considerations are needed for larger proteins. From a preformulation perspective, the goals are the same—to characterize the drug compound and understand the solubility and stability of the drug as well as the interactions with potential excipients that would be used to formulate the drug compound. Early results may determine the formulation strategy of either a ready-to-use solution or a lyophilized product for reconstitution. On the basis of this strategy, additional preformulation studies may be needed to support the formulation choice.

Characterization

In addition to the conventional characterization described earlier in the chapter, additional parameters relevant to protein drugs need to be assessed (50). These include determination of molecular weight, amino acid sequence, and disulfide bonds. Because of a large number of charged groups, proteins are generally soluble in water but can be physically unstable at high concentrations because of their complex interaction with surrounding water. Proteins are zwitterionic in nature as a consequence of the amino and carboxylic groups of individual amino acids. At low pH values, proteins would have a net positive charge, and at higher pH values, due to ionization of the carboxyl groups, they carry a net negative charge. The isoelectric point, pI, is the pH of an aqueous solution of a peptide (or protein) at which the molecules on average have no net charge. In other words, the positively charged groups are exactly balanced by the negatively charged groups. This is an important parameter, which is most commonly determined using an electrophoresis technique called isoelectric focusing.

From a solid-state point of view, protein drugs are frequently amorphous and quite hygroscopic. For large proteins made by genetic engineering technologies, it is also quite common not to routinely isolate the protein as a solid but to hold it in a solution or frozen buffered and stabilized solution.

Stability

The pharmacological activity of proteins and peptides is largely dependent on their intact primary, secondary, tertiary, and quaternary structures. Proteins and peptides are quite fragile and can undergo physical and chemical degradation under a variety of conditions.

Chemical Stability

Chemical degradation can be triggered by changes in temperature, pH, oxygen levels, and trace metals and under the influence of light. Methionine, cysteine, tryptophane, and histidine residues can undergo oxidation under the influence of trace metals and light and higher levels of oxygen. Hydrolysis of the side chains of asparagine and glutamine residues can result in deamidation reaction. Hydrolysis of the amide bond in the protein backbone is another degradation route, which is mainly influenced by the solution pH. β-elimination of cysteine, serine, threonine, and lysine residues is also affected by the solution pH, temperature, and ionic composition.

To characterize the degradation pathways, a multitude of analytical techniques are employed. These include different sequencing (*N*-terminal sequencing), spectroscopic (UV spectral analysis), separation (e.g., ion exchange, reverse phase, gel electrophoresis with protein staining, isoelectric focusing) of the intact proteins or enzymatically digested proteins (peptide map), and mass spectroscopic analysis of proteins to define the chemical modifications occurring. Circular dichroism is used to assess secondary and tertiary structures.

Physical Stability

Native protein structures are not very thermodynamically stable. Proteins easily unfold (denaturation) under the influence of increased temperature and concentration, pH change, buffer species, or chemical and physical stress. Completely or partially unfolded proteins can associate to form irreversible aggregates. Aggregation is not necessarily visible to the eye, but with increasing aggregation, aggregate size increases, and eventually, precipitation can occur, which is clearly visible.

Fluorescence measurements, light scattering techniques (sometimes in combination with reverse-phase or size exclusion chromatographic separation) and field flow fractionation can be used to assess aggregation. Conformational changes leading to aggregation can also be measured by DSC.

Protein unfolding, adsorption to surfaces, and aggregation can be modulated by pH, buffer species, choice of preservatives, and use of appropriate surfactants and stabilizers (sugars) in the formulation. The formulation factors have to be tailored to individual proteins through well-executed studies evaluating formulation, processing, and storage conditions. Other chapters in this book cover protein characterization and formulation aspects in detail.

SUMMARY

The aim of preformulation studies is to gain a thorough understanding of the drug molecule, its physical and chemical properties, as well as its interaction with other formulation ingredients and packaging materials to drive a rational formulation design. This chapter has provided an overview of preformulation studies related to development of parenteral medications.

REFERENCES

1. Halbert GH. Preformulation. In: Florence AT and Shipman J, ed. Modern Pharmaceutics, Basic Principles and Systems. Vol. 1. New York: Informa Healthcare, 2009:327–355.
2. Gardner CR, Walsh CT and Almarsson O. Drugs as materials: valuing physical form in drug discovery. Nat Rev Drug Discov 2004; 3:926–934.
3. Bhattachar SN, Deschenes LA and Wesley JA. Solubility: it's not just for physical chemists. Drug Discov Today 2006; 11(21/22):1012–1018.
4. Anderson BD and Flora KP. Preparation of water soluble compounds through salt formation. In: Wermuth CG, ed. The Practice of Medicinal Chemistry. London: Academic Press, 1996:739–754.
5. Yalkowsky SH and Roseman TJ. Solubilization of drugs by cosolvents. In: Yalkowsky SH, ed. Techniques of Solubilization of Drugs. New York: Marcel Dekker, 1981:91–134.
6. Yalkowsky SH, Kryzyaniak JF and Ward GH. Formulation related problems associated with intravenous drug delivery. J Pharm Sci 1998; 87(7):787–796.
7. Yalkowsky SH, Valvani SC and Johnson BW. In vitro method for detecting precipitation of parenteral formulations after injection. J Pharm Sci 1983; 72(9):1014–1017.
8. Davis ME and Brewster ME. Cyclodextrin-based pharmaceutics: past, present and future. Nat Rev Drug Discov 2004; 3:1023–1035.
9. Brewster ME and Loftsson T. Cyclodextrins as pharmaceutical solubilizers. Adv Drug Del Rev 2006; 59:645–666.
10. Stella VJ and Rajewski RA. Cyclodextrins: their future in drug formulation and delivery. Pharm Res 1997; 14(5):556–567.
11. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research. Guidance for industry Q3B(R2) impurities in new drug products. Available at: http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm128033.pdf. Accessed December 2009.
12. European Medicines Agency. Evaluation of medicines for human use. Available at: http://www.emea.europa.eu/pdfs/human/swp/519902en.pdf. Accessed December 2009.
13. Alsante KM, Ando A, Brown R, et al. The role of degradant profiling in active pharmaceutical ingredients and drug products. Adv Drug Del Rev 2006; 59:29–37.
14. Baertschi SW. Analytical Methodologies for discovering and profiling degradation-related impurities. Trends Anal Chem 2006; 25(8):758–766.
15. Stella VJ. Chemical and physical bases determining the instability and incompatibilities of formulated injectable drugs. J Paren Sci Technol 1986; 40(4):142–163.

16. Waterman KC, Adami RC, Alsante KM et al. Hydrolysis in pharmaceutical formulations. Pharm Dev Technol 2002; 7(2):113–146.
17. Won CM, Molnar TE, Windisch VL et al., Kinetics and mechanism of degradation of klerval, a psuedo-tetra peptide. Int J Pharm 1999; 190:1–11.
18. Zhu H, Meserve K and Floyd A. Preformulation studies for an ultrashort-acting neuromuscular blocking agent GW280430A. I. Buffer and cosolvent effects on the solution stability, Drug Dev Ind Pharm 2002; 28(2):135–142.
19. Wasylaschuk WR, Harmon PA, Wagner G, et al. Evaluation of hydroperoxides in common pharmaceutical excipients. J Pharm Sci 2007; 96(1):106–116.
20. Won CM, Tang SY, Strohbeck CL. Photolytic and oxidative degradation of an antiemetic agent, RG 12915. Int J Pharm 1995; 121:95–105.
21. Tonnesen HH. Photodecomposition of drugs, In: Swarbrick J, ed. Encyclopedia of Pharmaceutical Technology. 3rd ed. New York: Informa Healthcare, 2006:2859–2865.
22. Cosa G. Photodegradation and photosensitization in pharmaceutical products: assessing drug phototoxicity. Pure Appl Chem 2004; 76(2):263–275.
23. Li YNB, Moore DE, Tattam BN. Photodegradation of amiloride in aqueous solution. Int J Pharm 1999; 183:109–116.
24. Lutka A. Effect of cyclodextrin complexation on aqueous solubility and photostability of phenothiazine. Pharmazie 1997; 55:120–123.
25. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, and Center for Biologics Evaluation and Research. Guidance for industry Q1B photostability testing of new drug substances and products. Available at: http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm073373.pdf. Accessed December 2009.
26. Serajuddin ATM. Salt formation to improve drug solubility. Adv. Drug Del Rev 2007; 59:603–616.
27. Agharkar S, Lindenbaum S, Higuchi T. Enhancement of solubility of drug salts by hydrophilic counterions: properties of organic salts of an antimalarial drug. J Pharm Sci 1976; 65:747–749.
28. Tong W-Q and Whitesell G. In situ salt screening-a useful technique for discovery support and preformulation studies. Pharm Dev Technol 1998; 3(2):215–223.
29. Cavatur RK, Vemuri NM and Suryanarayanan R. Preformulation studies for tablet formulation development. In: Hoag SW and Augsberger LL, ed. Pharmaceutical Dosage Forms: Tablets Vol. 1. Unit Operations and Mechanical Properties. New York: Informa Healthcare, 2007:465–483.
30. Vemuri NM, Chrzan Z and Cavatur R. Use of isothermal microcalorimetry in pharmaceutical preformulation studies part II: amorphous phase quantification in a predominantly crystalline phase. J Thermal Anal and Calorimetry 2004; 78:55–62.
31. Nema S, Washkuhn RJ and Brendel RJ. Excipients and their use in injectable products. PDA J Pharm Sci Technol 1997; 51(4):166–171.
32. Nema S., Brendel RJ and Washkuhn RJ. Excipients: parenteral dosage forms and their role. In: Swarbrick J, ed. Encyclopedia of Pharmaceutical Technology. 3rd ed. New York: Informa Healthcare, 2006:1622–1645.
33. U.S. Food and Drug Administration. Inactive ingredient search for approved drug products. Available at: http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm. Accessed December 2009.
34. Akers MA. Excipient-drug interactions in parenteral formulations. J Pharm Sci 2002; 91(11): 2283–2300.
35. Durig T and Fassihi R. Identification of stabilizing and destabilizing effects excipient-drug interactions in solid dosage form design, Int J Pharm 1993; 97:161–170.
36. Wissing S, Graig DQM, Barker SA et al. An investigation into the use of stepwise isothermal high sensitivity DSC as a means of detecting drug-excipient incompatibility. Int J Pharm 2000; 199:141–150.
37. Phipps MA and Mackin LA. Application of isothermal microcalorimetry in solid state drug development. Pharm Sci Technol Today 2000; 3(1):9–17.
38. Cavatur R, Vemuri NM and Chrzan Z. Use of isothermal microcalorimetry in pharmaceutical preformulation studies, III: evaluation of excipient compatibility of a new chemical entity. J Thermal Anal and Calorimetry 2004; 78:63–72.
39. Peswani KS and Lalla JK. Naproxen parenteral formulation studies. J Parent Sci Technol 1990; 44(6):336–342.
40. Lewis GA, Mathieu D and Luu RPT. Screening. In: Pharmaceutical Experimental Design. New York: Marcel Dekker, 1999:23–78.
41. Gupta SL, Patel JP, Jones DL et al. Parenteral formulation development of renin inhibitor Abbott-72517. J Paren Sci Technol 1994; 48(2):86–91.
42. Wong J, Brugger A, Khare A et al. Suspensions for intravenous injection: a review of development, preclinical and clinical aspects. Adv. Drug Del Rev 2008; 60:939–954.

43. Boyd BJ. Past and future evolution in colloidal drug delivery systems. Expert Opin Drug Del 2008; 5(1):69–85.
44. Merisko-Liversidge E, Liversidge GG and Cooper ER. Nanonizing: a formulation approach for poorly-water-soluble compounds. Eur J Pharm Sci 2003; 18:113–120.
45. Rabinow BE. Nanosuspensions in drug delivery. Nat Rev Drug Disc 2004; 3:785–796.
46. Euliss LE, DuPont JA, Gratton S. et al. Imparting size, shape, and composition control of materials for nanomedicine. Chem Soc Rev 2006; 35:1095–1104.
47. Prankerd RJ and Stella VJ. The use of oil-in-water emulsions as a vehicle for parenteral drug administration. J Paren Sci Technol 1990; 44(3):139–149.
48. Constantinides PP, Lambert KJ, Tustian AK et al. Formulation development and antitumor activity of a filter sterelizable emulsion formulation of paclitaxel. Pharm Res 2000; 17(2):175–181.
49. Driscoll DF. Lipid injectable emulsions: pharmacopeial and safety issues. Pharm Res 2006; 23(9): 1959–1969.
50. Nguyen LT, Wiencek JM and Kirsch LE. Characterization methods for the physical stability of biopharmaceuticals. PDA J Paren Sci Technol 2003; 57 (6):429–445.