Chapter 13

Formulation Development of Parenteral Products

Objectives

This chapter provides an overview of the development of injectable (parenteral) drug products. Injectable drug products are relatively specialized and diverse, depending on both the location and type of disease to be treated in a patient. Developing an optimized formulation around a certain type of product will determine not only whether or not the drug will be effective for its intended use, but also if it will be stable for an extended period of time. Pre-formulation studies are used to both determine the physical properties of a drug molecule of interest and understand the conditions where the drug is stable. Formulation development studies achieve those optimal conditions through either the use of additives or the manner in which the drug is processed. Additionally, since the body's natural defenses are bypassed when injecting this type of drug product, special care must be taken to ensure that micro-organisms and other extraneous materials are not present.

After completing this chapter the student will be able to:

- define the different types of injectable drug products.
- understand the different specifications required for injectable drug products.
- understand the process of developing an optimized formulation in a laboratory.
- understand the process of transferring and scaling up the new formulation to a large-scale manufacturing environment.

Terms

Buffer Capacity: the strength of a buffer system; the amount of acid or base that can be added before the solution pH shifts

Buffer System: a weak acid and its conjugate salt that prevents the pH from shifting in a solution

Emulsion: in colloid chemistry, a suspension of one liquid (oil) in another (aqueous)

Endotoxin (Pyrogen): a lipopolysaccharide molecule buried in the cell wall of Gram negative bacteria; when these cells die, they release the endotoxin, which is highly toxic to humans and animals (sepsis, septic shock).

Flocculation: in colloid chemistry, refers to the process by which fine particulates are caused to clump together into a floc

Parenteral: a route of administration of a substance into the body other than through the mouth, eyes, or skin

Process Compatibility: testing performed in order to understand the interaction of an active pharmaceutical ingredient in a formulated product with components that it contacts during manufacturing

Stability Indicating Method: an analytical method that can be used to accurately and precisely determine the degradation of the drug product

Surfactant: a molecule that has both "water loving" and "water hating" ends (surface active); these molecules change the surface tension of water and preferentially bind to surfaces to prevent molecules of interest from sticking to those surfaces or to each other.

Suspension: insoluble particles suspended in a liquid (vehicle)

Introduction to Parenteral Products

Basic theory

Parenteral (*para enteron*—beside the intestine) administration is the introduction into the body of nutrition, medications, or other substances other than by the alimentary canal. Unlike taking medications orally or rectally, introducing a drug into the body by parenteral administration poses far greater risk since the body's natural defenses are bypassed. As such, they must be exceptionally pure and free from physical, chemical, and biological contaminants. These conditions place a huge burden on pharmaceutical companies that manufacture these types of products, as the regulations enforced by the Food and Drug Administration (FDA) are extremely stringent.

Drugs are administered parenterally for a number of different reasons. The first and foremost reason is that if the drug is administered by the intravenous route (IV), then there is an immediate onset of pharmacological action, unlike tablets which take approximately 45 minutes to have an effect once taken. This is extremely important in pain management or in the case of a psychotic patient who needs immediate treatment with an anti-psychotic agent. Also, unlike tablets, where a certain amount of the drug is lost due to the body breaking it down or removing it from the bloodstream, drugs administered by IV are 100 percent bioavailable to the body. Drugs are also administered by the parenteral route if a targeted part of the body needs treatment. For example, if a patient is having issues with a knee, drugs can be injected directly into the knee for maximum effect. This technique also allows for some of the more toxic drugs to be targeted to specific sites without contaminating the bloodstream. Finally, drugs are administered parenterally because they would be destroyed if administered orally. Many of the new drugs coming out of discovery are biologically based (proteins, monoclonal antibodies, etc.), and the body simply sees these drugs as another source of protein if taken orally and releases enzymes to break them down for food. For these types of products, parenteral administration is the only option for administration, although some can be applied topically. The drawbacks to parenteral products are as follows:

- pain on injection

- site must be sterilized before administration

- bypasses the body's natural defenses against infection

- cannot be taken back once administered (e.g., wrong drug administered)

There are several different parenteral formulations that are available for parenteral administration; they are as follows:

- solutions ready for injection

- dry, soluble products (freeze-dried or powder fill) ready to be combined with a solvent prior to administration

- suspensions ready for injection

- dry, insoluble products ready to be combined with a solvent prior to administration

- emulsions
- liquid concentrates ready for dilution prior to administration

Developing these different types of formulations depends on a number of factors, including route of administration, area of administration, onset of action, rate of drug release, and shelf life.

Brief history

Drugs were being administered intravenously into animals as early as 1657 with mixed results. Over the next 150 years, the science of injectable drugs slowly developed, with the first use of a hypodermic needle being recorded in 1855 by Dr. Alexander Wood in Edinburgh to administer a therapeutic drug subcutaneously (under the skin) to a human patient. While the knowledge of therapeutic drugs was available to Dr. Wood at that time, the issue of sterility and micro-organisms was not as well understood, and patients were dying from complications due to impurities, non-sterility, and/or endotoxin contamination (pyrogens). It was not until the 1920s, due to the work of several researchers, including Pasteur, Koch, Chamberlin, and Limousin, that an understanding of micro-organisms and sterilization practices came into place, making these types of drugs safer for administration.

Early in the history of injectable drug products, sterilization of the product in vials was accomplished using super-heated, saturated steam (autoclaving). Many of the new drugs coming out of discovery today, such as proteins and monoclonal antibodies, cannot tolerate the high temperature of steam sterilization, so they are manufactured through aseptic manufacturing, in which all of the components are pre-sterilized and then brought together in a sterile environment. Stringent controls and testing are required with this manufacturing technique as there is no absolute assurance of sterility. The two largest technical developments allowing aseptic manufacturing to occur effectively have been the development of High Efficiency Particulate Air (HEPA) filters and membrane filters for sterile filtration of the injectable solutions.

Injectable Solutions

Injectable solutions are those products in which the drug, any added excipients, and any added co-solvents all truly dissolve in the vehicle (usually water). This type of presentation is the most common of the injectable drug products, and these types of products have the potential to be injected by any route of administration into the body. Parenteral suspensions and emulsions will be introduced and discussed below; however, these formulations are extremely complex and difficult to formulate, so the majority of this chapter is based on the injectable solution presentation. An image of an injectable solution is depicted in Figure 13-1.

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Figure 13-1. An injectable solution

Injectable Suspensions

Suspensions are products where the active ingredient is extremely insoluble in the solution used to deliver it (the vehicle). Suspensions are formulated as such so when they are injected to a precise location, the drug crystals slowly dissolve, resulting in a controlled, slow release of the drug to the surrounding capillaries where it is absorbed into the bloodstream. The route of administration is typically intramuscular (IM), but they have also been administered by the subcutaneous, intradermal, intralesional, or intra-articular routes. Suspensions can never be administered by the IV route, as the drug crystals would block capillaries and cause necrosis of the tissues.

Suspensions are complex formulations both in how they release drug to the body and how they are formulated for physical characteristics in the vial. For example, if the formulation is not properly designed, the drug crystals can sink to the bottom and form a hard layer referred to as concretion. If allowed to sit long enough, this solid layer is extremely difficult or impossible to re-suspend. Some amount of settling always occurs in a suspension; however, it is the job of the formulation scientist to develop a formulation that prevents concretion and allows the suspension to re-suspend easily with moderate shaking.

There are three different approaches that can be used to keep solid particles suspended in a liquid:

- Stokes's law

- flocculation

- structured vehicle

Stokes's law: getting solids to suspend by slowing the velocity with which the solids settle in the liquid—according to Stokes equation below:

$$v = \frac{d^2(\rho_s - \rho_l)g}{18\eta}$$

where: v = sedimentation velocity (cm/sec)
d = diameter of particle (cm)
ρ_s = density of solid phase
ρ_l = density of liquid phase
g – acceleration due to gravity (980 cm/sec^2)
η = viscosity, in poise

Referring to the equation, there are several ways in which to slow the settling of the solids. Decreasing the size of the solids, adjusting the density of the solid phase to that of the liquid phase (if too far off, the solids will either sink or float in the liquid phase), and/or increasing the viscosity of the liquid phase have all been used to prepare suspensions using the Stokes's law principle.

Flocculation

The flocculation approach for suspending solids in a liquid phase is based on the repulsive and attractive forces of charged species. The goal of the development scientist developing a suspension using the flocculation approach is to make the solids repulse each other when they get too close but attract each other when they are at a distance. Figure 13-2 depicts a flocculated suspension when fully suspended and when settled. Even when settled the solids are still flocculated and re-suspend with minimal shaking.
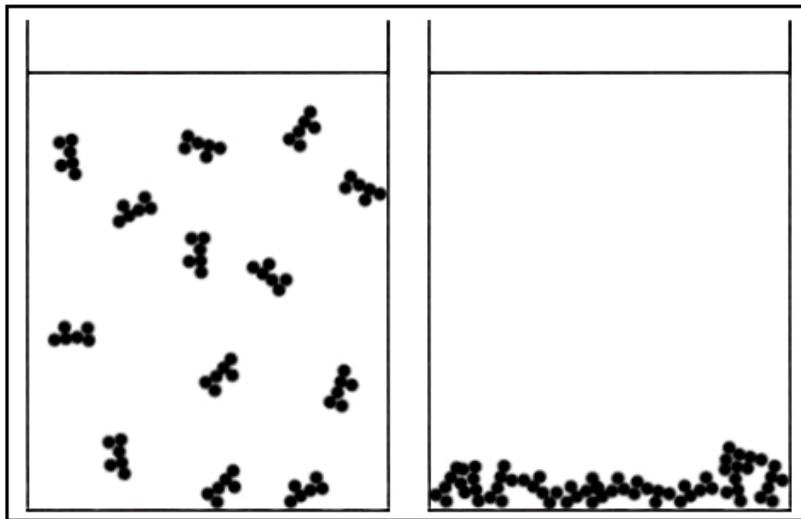

Figure 13-2. Flocculated suspension

Structured vehicle

The structured vehicle approach is based on the viscosity of the suspending solution (vehicle) and how the viscosity changes with shear force (shaking).

The study of viscosity (Rheology) is an involved science, and liquids behave differently depending on their characteristics. Many fluids follow what is known as Newtonian Flow, which states that the shear stress (force per unit area applied) is proportional to the rate of shear (flow of the system as a result of applying shear stress). As increased shear stress is applied, the rate of shear increases linearly for Newtonian fluids. In a well-designed suspension the vehicle experiences Non-Newtonian Flow. Certain fluids experience Non-Newtonian, shear-thinning characteristics, while others experience shear-thickening characteristics. Shear thickening liquids become extremely thin and watery but get thick when shaken. Shear thinning liquids become viscous when sitting still but become thin when shaken. The shear thinning characteristics of materials make them ideal for suspensions. In more practical terms, quicksand is a shear thinning material, which is what makes it dangerous—the struggle to get out causes the quicksand to become thinner, resulting in the person or animal trapped in it to sink quicker.

Suspensions utilizing the structured vehicle approach contain certain polymers or charged clays. When allowed to sit undisturbed, these materials quickly form a robust, three-dimensional structure strong enough to hold the suspended solids and keep them from settling to the bottom. In the case of the polymers, the polymer chains intertwine, and in the case of the clays, the individual particles have different charges on the ends versus the charges on the body. These different charges attract other particles and form something similar to a house of cards when allowed to sit undisturbed. These bonds are easily broken with shaking, allowing the suspension to become thin so that the solids can be easily re-suspended.

Injectable Emulsions

If an injectable drug is desired with a rapid onset of action but the drug is extremely insoluble in water, an emulsion might be a suitable alternative. Emulsions can be considered oil in water (o/w) or water in oil (w/o) emulsions, meaning either small oil droplets in a water base or small water droplets in an oil base respectively. An injectable emulsion is depicted in Figure 13-3.

Figure 13-3. An injectable emulsion

Many emulsions are used cosmetically or as pharmaceutical creams to be administered topically on the skin. For injectable emulsions, the o/w form is traditionally used. In these types of products, the poorly water soluble drug is easily dissolved in oil (soybean, cottonseed, peanut, etc.) and then suspended using high shear stress to turn the oil into fine droplets suspended in the water base.

Like suspensions, emulsions tend to be complex formulations and require a high level of engineering to be formulated correctly. One of the key components required for a properly formulated suspension is the emulsifying agent. The emulsion must be stable, meaning that once the fine oil droplets are produced they need to remain as droplets at the same size. One way to envision this is if oil, water, vinegar, and spices are mixed together to form salad dressing. When shaken vigorously the mixture takes on a uniform appearance; however, if allowed to sit undisturbed, the oil and water phases separate and form two layers, with the less dense oil floating on top of the water. The emulsifying agent prevents this phase of separation from occurring. These emulsifying agents are referred to as surfactants, meaning one end of the molecule has a water-loving end, and the other end of the molecule has an oil-loving end. An example of a surfactant molecule widely used in injectable emulsions is phosphatidylcholine (Figure 13-4). This surfactant comes from the yellow portion of chicken eggs. The green and blue portions of the molecule represent the greasy, oil-loving end of the molecule, and the red portion represents the charged water-loving end of the molecule.
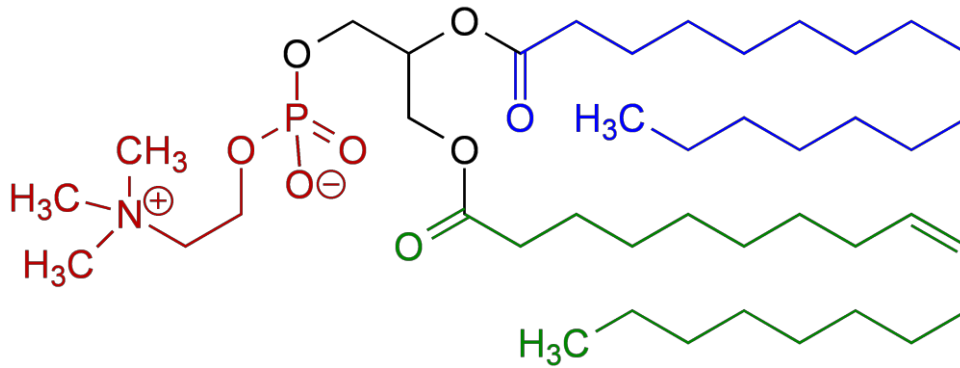
Figure 13-4. Phosphatidylcholine surfactant molecule

When the fine oil droplets are created through a high shear mixer or other device, the surfactant surrounds the oil droplets with the oil-loving tails toward the oil and the water-loving end on the outside. This prevents the oil droplets from coming together and forming a separate oil layer above the aqueous phase.

To get the oil phase (containing the active ingredient) to form the fine droplets, a significant amount of force must be applied. A specialized piece of equipment known as a Microfluidizer uses a pump to significantly increase the pressure up to 40,000 psi. The high pressure mixture is then forced through an interaction chamber, which shears the oil into micron-sized droplets. Figure 13-5 illustrates the process of forming the oil droplets.
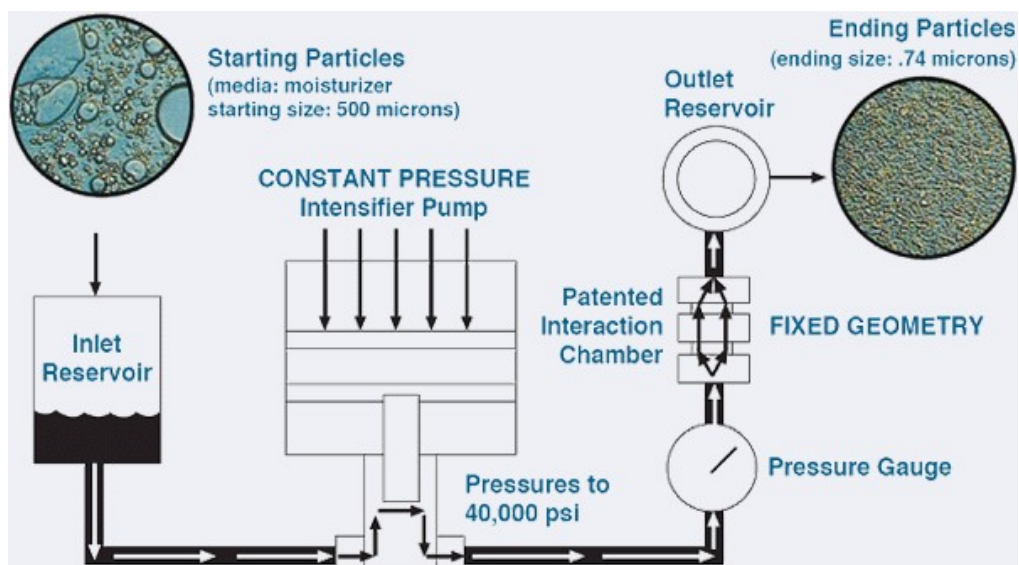

Figure 13-5. Oil droplet formation
(courtesy Microfluidics)

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Routes of Administration

A parenteral product may be administered via several different routes based on different criteria, including onset of action, bioavailability, and delayed release. The most common routes of administration are:

- subcutaneous – under the skin

- intramuscular – into a muscle

- intravenous – into a vein either by bolus (directly into a vein) or infusion (diluted in a bag of saline or dextrose and slowly administered over time)

There are other routes of administration, including injection into the brain or spinal column, the eye, and arteries. These types of products, however, require special care and expertise in formulation as these areas of the body are extremely sensitive and are easily damaged if products are not formulated properly.

Specifications

Injectable drug products, like other drug products, must meet certain specifications in order to be released for sale to the public. Unlike oral drugs (tablets, capsules, etc.), injectable drug products have additional specifications that must be met due to these products bypassing the body's natural defenses against microorganisms. Mandatory attributes of injectable drug products are:

- sterility – free from yeasts, molds, bacteria, viruses, etc.

- particle free – must be free of particles over a certain size (unless a suspension)

- endotoxin free - a highly toxic molecule coming largely from the cell wall of dead, gram-negative bacteria

Additional specifications that must be met (dependent on the product) include:

- appearance

- pH – try to get as close to the body's natural pH

- potency

- purity

- tonicity – the salt/ionic balance (try to get as close to the body's natural tonicity)

- specialized specifications (proteins, monoclonal antibodies, vaccines, etc.)

Special care must be given to ensure that products are free of endotoxin or pyrogens. The term endotoxin was coined several years ago and is actually a misnomer. Early researchers noted that when gram-negative bacteria died they released a toxin; they assumed it was coming from inside the cells. Endotoxins, however, are actually exotoxins, as they are in the outer cell wall of these bacteria.

Endotoxin molecules are lipopolysaccharides, which are composed basically of a lipid end (fat) and a polysaccharide end (sugar chains). Studies have shown that the lipid end of the molecule is responsible for the toxicity. When a gram-negative bacterial cell is alive, the lipid end of the molecule is buried in the hydrophobic interior of the cell wall, while the sugar chains are extending outside the cell. When the lipid end is buried it cannot exert its toxic effect; however, when these cells die the lipid end of the molecule is exposed and becomes toxic.

When introduced into the body by injection, small amounts of endotoxin can cause fever, chills, pain in the back and legs, and malaise. Higher amounts lead to septic shock (i.e., toxic shock) and can lead to death; therefore, testing is critical to ensure products to be injected are endotoxin free. In most cases endotoxin cannot be removed from a product once present, so care must be taken to ensure that all of the components, including active ingredients, excipients, vials, and stoppers, are free of endotoxin and that anything that comes in contact with the product is endotoxin free. Endotoxin can be destroyed with high heat (tunnel ovens heat vials to sterilize and remove endotoxin prior to filling product), sodium hydroxide (stoppers can be washed in high pH solutions), and hydrogen peroxide.

In the past, testing for endotoxin contamination in products was a time-consuming and difficult process, and the results were often variable. In the early 1970s, endotoxin testing was conducted using rabbits. A rabbit would be fitted with a thermocouple to monitor its temperature; samples from the drug product batch would then be administered; and the rabbit would be monitored for fever. Rabbits were chosen due to the large veins in their ears, which made administration easier. Substantial amounts of time and effort went into the testing, including training the rabbits for the testing, housing them, and feeding them. Additionally, the rabbits could only be used once, and then they were destroyed. This was not much cause for concern in the '70s but would likely be an issue today in regards to animal testing requirements and the ethical treatment of animals.

In the mid-1950s, a scientist discovered that when the blood from a horseshoe crab was mixed with dead, gram-negative bacterial cells it would clot. This is a defense mechanism of the horseshoe crab that allows it to prevent infections from spreading through its circulatory system if breached. Over the years scientists were able to determine that this was due to a clotting factor within the blood cells. By extracting blood from horseshoe crabs and lysing the cells this clotting factor was released. This factor could then be collected and freeze-dried into a stable powder. To achieve this, horseshoe crabs are collected, their hearts are pierced with a needle, and a certain volume of blood is removed and used to extract the clotting factor. This test is known as the Limulus amebocyte lysate test or LAL testing. (It should be noted that in most cases the horseshoe crabs are returned to the wild without any long-term ill effects.) There are several different types of the LAL test that are used, including:

- chromogenic – this method uses a chromophore (detected by UV/Vis) which is cleaved by the endotoxin enzyme

- turbidimetric – (most widely used) this method detects the turbidity (cloudiness) of the solution over a period of time as the clots form in the presence of endotoxin

- <u>gel clot</u> – this method is a simple *yes* or *no* answer and does not determine the amount of endotoxin like the previous methods

Formulation Development

Introduction

Formulation of a stable, safe, and effective injectable drug product is a sizable, challenging task involving many scientific disciplines and encompassing years of research and development. Once a drug is discovered and the first patent is filed, the company holding the patent has 17 years of market exclusivity for the drug before generic drug companies can begin selling the drug product. On average it takes approximately 10 years of work in areas such as development, clinical trials, and FDA approval before a drug can be sold to the public, which means that a company only has seven years to recoup all of their development costs (approximately $1 billion) and make a profit on the drug product. This time period begins once the patent is filed and the formulation development process begins. An additional upstream phase of development, not covered in this chapter, is the development of a process for bulk product manufacturing, including bulk drug powder and protein slurry. A significant amount of work goes into this phase of development and is out of the scope of this chapter. It is assumed that this work has been completed prior to starting formulation development studies, and the development scientist has access to the bulk drug powder for use in the studies. It is the responsibility of the formulation development scientist to take the bulk drug and develop ways to dissolve it, stabilize it, and ensure that it is safe and effective for administration to the end user.

The formulation development process can be broken down into several distinct phases. These phases are:

- pre-formulation assessment
- pre-formulation development
- formulation development
- process development
- process compatibility
- scale-up

Each of these phases plays a critical role in the development of a drug product, and the information learned in one phase drives the development of the next phase. It must be understood that the final formulation is not developed until later in the development phase, but that is not to say that injectable formulations are not needed early on in the process. As the drug goes through the early development phases, studies are needed to determine the effects of administration first into animals and then into humans, and this information is needed long before the final formulation is developed. The FDA understands this and allows flexibility in tweaking the formulation to meet these needs. In fact, the formulation, manufacturing process, and the analytical methods may all be altered to meet the needs for the clinical trials up to phase III. Once material is to be made for phase III clinical trials, all must be in place and

validated prior to making product for these studies, including the final formulation, analytical techniques, and manufacturing process. If changes are made after this study, companies are required to repeat trials and stability studies. This can be expensive and can delay the drug entering the market, resulting in a significant loss in revenue.

The development process described below is a typical protocol for developing a formulation for a small, crystalline molecule. The process gets more complicated both in the formulation development and the analytical development when large biomolecules are the active ingredients, including large proteins and monoclonal antibodies.

Pre-formulation assessment

The pre-formulation assessment is an information gathering process to ensure that all of the required information is in place to begin the pre-formulation development studies. Having all of this information in place before starting pre-formulation development studies saves the development scientist both time and effort. Much of the information needed for the pre-formulation development studies can be taken from the development work on the bulk drug itself and includes:

- <u>stability indicating analytical method</u> – This is the most important piece of information to have prior to beginning development studies. Unless the development scientist can detect if the drug is not breaking down during the development process, it is useless to proceed.

- <u>thermal stability</u> – Does the bulk drug degrade in response to high temperatures?

- <u>oxidation potential</u> – Does the drug degrade when exposed to oxygen?

- <u>light stability</u> – Does the drug degrade in the presence of light?

- <u>bulk drug preparation</u> – How is the bulk drug made, and are there any residual solvents present from the manufacturing process?

- <u>polymorph existence</u> – Do other crystalline forms of the bulk drug exist?

- <u>pKa or pI</u> – This is the acid dissociation constant for small molecules or the isoelectric point for proteins. This provides information about the solubility of the bulk drug.

Pre-formulation development

The pre-formulation studies are designed essentially to find the conditions in which the molecule is most stable. Once these conditions are understood, the goal of the formulation development studies is to achieve these conditions through either the addition of excipients or how the product is processed (i.e., lyophilization). Pre-formulation development studies include:

Solubility

The solubility of the drug can be a challenge for the development scientist. In some cases a bulk drug simply floats on top of the water used to dissolve it. In this case, solubility studies must be conducted. There are several ways to enhance the solubility of poorly soluble compounds; when doing so, the following questions should be addressed:

- Can a salt be made of the free base?

- Is the correct pH being used?

- What are the effects of added co-solvents?

- What are the effects of added complexing agents?

Molecules in solution can be either uncharged (free base or free acid) or charged (ionic salt forms). Generally the salt forms are more soluble than the uncharged species, so by creating an ionic salt form of the molecule the solubility is enhanced. This is typically the first and easiest way to enhance the solubility of poorly soluble compounds.

The solution pH can have a significant impact on the solubility of poorly soluble compounds. As the solution pH is changed, different species on the molecule are either protonated (adding a hydrogen ion) or deprotonated (taking away a hydrogen ion). This alters the charge on the molecule changing the solubility in water. To determine the effects of pH on solubility, a pH screening study can be initiated. For these studies, solutions can be prepared at different pH values, and the insoluble drug is added to the point of saturation (undissolved drug present).

Samples are removed, the undissolved drug is filtered out of the solution, and the drug concentration in solution is measured using an analytical technique (HPLC or UV/Vis). Using the resulting data, a graph can be generated showing the solubility as a function of pH. Figure 13-6 illustrates a solubility profile of procaine penicillin as a function of pH overlapped with a stability profile. The solubility of the penicillin increases significantly as the pH of the solution is lowered (acidified); however, the stability of the molecule is greatly reduced as well. This is generally the case with altering the pH to enhance stability, and there needs to be a balance between solubility and stability of the molecule.
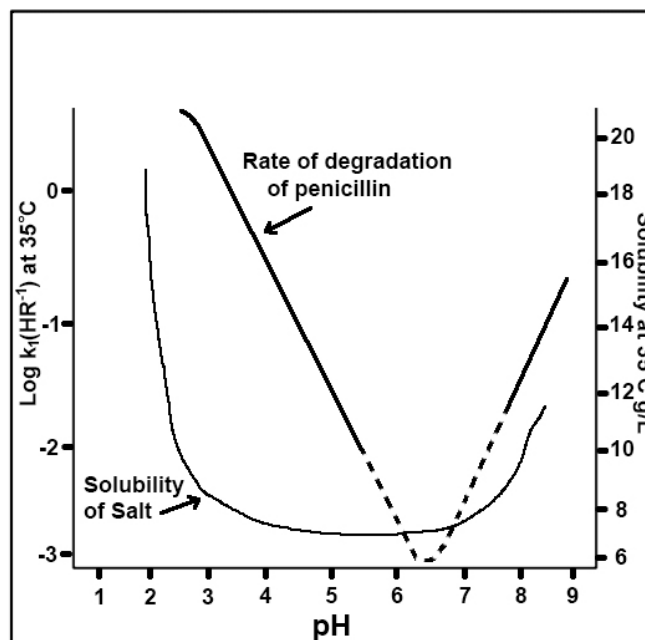

Figure 13-6. Solubility vs. stability curve

Co-solvents can have an impact on the solubility of poorly soluble compounds as well. According to the adage "like dissolves like," molecules with poor water solubility are more soluble in those solvents that are less like water. As such, a solvent screening study can be

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initiated. For these studies, various concentrations of different solvents are prepared, and the insoluble drug is added to the point of saturation (undissolved drug present). Samples are removed, the undissolved drug is filtered out of the solution, and the drug concentration in solution is measured using an analytical technique (HPLC or UV/Vis). Using the resulting data, a graph can be generated showing the solubility as a function of solvent concentration. A typical solvent solubility graph is depicted in Figure 13-7.
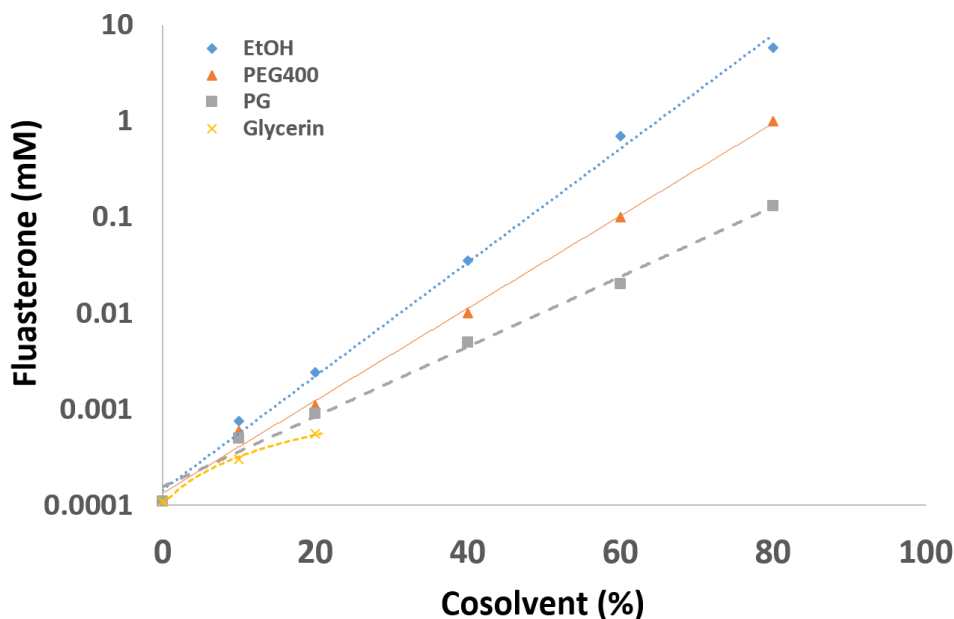

Figure 13-7. Co-solvent solubility curves

It must be understood that even though the solvent screening study shows that the best solubility is achieved at higher concentrations of solvent, there is a limit as to what can be injected safely into humans and animals.

Complexing agents can be used as a viable alternative if the aforementioned solubilizing techniques do not provide enough solubility. Complexing agents or inclusion complexes are formed when a macro-cyclic molecule having an intramolecular cavity interacts with a smaller molecule that can enter the cavity. In this case the larger molecule is referred to as the host, and the smaller molecule entering the cavity is referred to as the guest. A group of compounds known as cyclodextrins are extremely well suited to act as complexing agents. The structure of a β-cyclodextrin is depicted in Figure 13-8.

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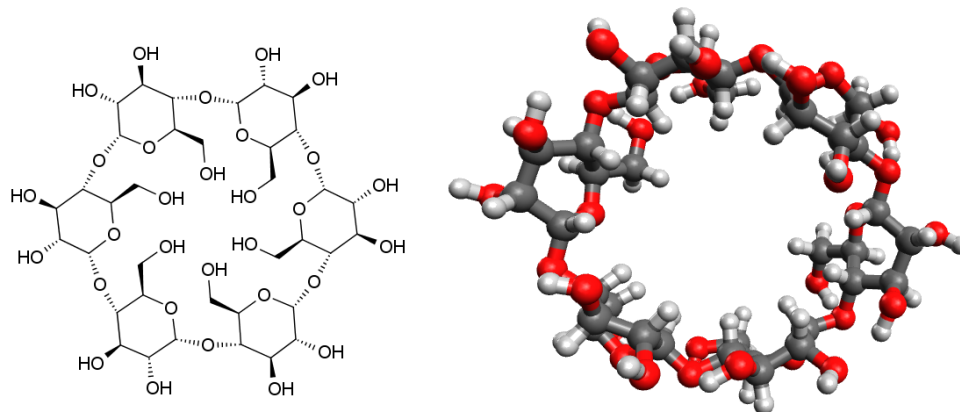
Figure 13-8. Cyclodextrin complexing agent

The interior of these molecules are hydrophobic (non-water loving), as is the poorly soluble guest molecule, so the guest is drawn into the cavity of the host. The outside of the host molecule is hydrophilic (water loving), so it is able to go into solution with the poorly soluble guest inside of it. When administered to the patient, the guest molecule is released from the host and is available for use by the body.

Degradation profile (stability)

Determining the degradation profile of the drug in the pre-formulation studies is something that provides a wealth of information when transitioning into the formulation development work. As mentioned previously, some of this information may come from the early work developing the bulk drug; if this is not the cases, however, this data should be generated as part of the pre-formulation development work.

The thermal stability of the bulk drug can be determined by placing samples of the bulk drug into a dark, nitrogen purged environment and exposing it to various high temperatures for extended periods of time. Typically samples are placed at 25°C, 40°C, 60°C, 80°C, and potentially even higher over a period of three to six months. Samples are pulled at regular intervals and tested for degradation using the stability indicating method described previously (typically HPLC for small molecules).

The stability as a function of pH should also be determined, as pH can have a dramatic impact on both the short- and long-term stability, as well as the solubility as described previously. This study is conducted by formulating the bulk in weakly buffered systems at various pH values and placing them at accelerated temperatures to speed up the results. Typically the first experiment involves formulating the bulk at pH 4, 5, 6, 7, 8, and 9 weakly buffered systems. Samples are tested initially for degradation impurities. They are then placed at 40°C or higher and tested

over several weeks to determine which pH value generated the least impurities over time. Based on these results, the pH solubility vs. stability graph can be generated (Figure 13-6) so that an informed decision can be made in choosing the best pH for good solubility and long-term stability.

Light stability of the molecule is determined using a light chamber (Figure 13-9).

Figure 13-9. Photo stability chamber
(courtesy Caron Products)

This unit controls the strength and wavelength of light that is exposed to the samples over time. The light stability of the bulk drug can be determined by placing samples of the bulk drug in a nitrogen-purged environment and exposing it to light for extended periods of time. Typically samples are placed in the light chamber and tested over a period of several months. Samples are pulled at regular intervals and tested for degradation using the stability indicating method described previously (typically HPLC).

The potential of the drug to oxidize is important to know, as many drugs are formulated and filled without any steps to minimize oxidation. If the potential for oxidation is known in advance, steps can be taken to reduce and/or eliminate oxygen from the samples during manufacturing. The potential for a drug to oxidize can be determined by exposing the drug to concentrated hydrogen peroxide and looking for the presence of degradation products using the stability indicating method (HPLC). Essentially a certain amount of drug is added to a concentrated solution of hydrogen peroxide and mixed. Over time samples are removed, neutralized with a reducing agent, and tested for degradation products. If the drug is easily

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oxidized, steps can be taken to prevent this (described in more detail in the Formulation Development section).

Formulation development

The goal of the formulation development work is to establish the optimal conditions determined in the pre-formulation development studies. These conditions are achieved through procedures such as the addition of excipients, the control of oxygen, and the control of hydrolysis (through lyophilization). Additional information regarding the drug product is needed before beginning formulation development work and includes the following:

- dose (number of mg of drug to be administered)
- route of administration (IM, IV bolus, IV infusion, SubQ, etc.)
- solution concentration (mg/mL)

In regards to what may be added to a drug product to help stabilize it or enhance its properties for injection, the FDA is strict and only allows certain materials to be added to injectable drug products. The best way to ensure that a certain excipient added to a drug product will not raise questions by the FDA is to use one from a previous drug product; use it at the same concentration (or less); and use the same route of administration. This information is available from several reliable resources. The FDA has a searchable website that lists pharmaceutically acceptable excipients, their concentrations, and the acceptable routes of administration. The website is searchable for each excipient as well and may be found at:

http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm

Another available resource for searching for acceptable excipients is the *Physician's Desk Reference*. This book contains a listing off all drugs on the market and includes their package inserts, which are documents containing extensive information about the drug products, including the excipients used (only for injectable drugs). There is a searchable electronic version of this resource, so a quick excipient search will locate all of the package inserts for the drugs containing that excipient.

Buffer Systems

If precisely controlling pH is a concern for product stability or solubility as determined in the pre-formulation studies, then the development of a buffer system may be necessary. A buffer system is a combination of a weak acid and its conjugate salt form that keeps the pH from shifting out of the optimal range when added acid or base gets into the system. There are several different types of buffer systems available; however, there are only a certain number that are deemed acceptable for injection. Below is a partial list of buffers commonly used for injectable drug products:

- phosphate (pKa = 2.1, 7.2, 12.7)
- citrate (pKa = 3.2, 4.8, 6.4)
- tris (pKa = 8.1)
- succinate (pKa = 4.2, 5.6)

- histidine (pKa = 1.8, 6.0, 9.0)

- glycine (pKa = 2.4, 9.8)

- arginine (pKa = 2.2, 9.1)

- malic (pKa = 3.4, 5.1)

- tartaric (pKa = 3.2, 4.8)

- acetic (pKa = 4.8)

- benzoic (pKa = 4.2)

- gluconic (pKa = 3.6)

- glyceric (pKa =3.55)

- lactic (pKa = 3.1)

- aconitic (pKa = 2.8, 4.5)

- adipic (pKa = 4.4, 5.3)

- ascorbic (pKa = 4.2, 11.6)

- carbonic (pKa = 6.4, 10.3)

- glutamic (pKa = 2.1, 4.3)

- ammonium chloride (pKa = 9.3)

- triethanolamine (pKa = 8.0)

The choice of buffer is determined by the optimal pH of the solution, which is determined in the pre-formulation studies. Each buffer system has at least one pKa value, which is the acid dissociation constant. This is the pH where half of the protons are removed from the molecules as the pH is increased. Buffers with more than one pKa value have more than one proton on the molecule to be removed. Buffer systems are strongest when they are formulated with the pH being the same value as one of their pKa values. This is logical because when the pH equals the pKa of the buffer, half of the buffer system is in the acid form (half have their hydrogen ions) and half is in the basic form (half have lost their hydrogen ions). When a slight shift up in pH occurs in the product, the acid form is there to neutralize it; and when a slight shift down in pH occurs, the basic form is there. A good rule of thumb to follow is to choose a buffer with a pKa that is within ± 1 unit from the desired solution pH.

Shifting of the pH in solution can come from several sources. As some drugs degrade they form carboxylic acids that lower the pH. Occasionally basic species from the glass vials can leach into the product and raise the pH. The buffer system is there to prevent the solution pH from shifting when these things occur.

Buffer strength is termed buffer capacity, and it is the buffer concentration that drives the buffer capacity—the amount of total acid or base the buffer can counteract before the buffer system is neutralized and the solution pH shifts. The rule of thumb to account for all of the acidic or basic factors over the lifetime of the drug is to add ten times the buffer based on 10

percent degradation of the drug over its lifetime. For example, if the active ingredient of a drug product is to be formulated at 10 mg/mL and the molecular weight of the drug is 200 g/mol, then the drug is not considered subpotent until 10 percent of the drug has degraded, which calculates to a loss of 0.005 moles/Liter (M). Therefore, adding a buffer at a concentration of 0.5 M would be appropriate. In fact, most injectable pharmaceutical buffer systems fall between 0.01 and 0.1 M, which makes the amount suggested in the example appropriate.

Buffer capacity is easily determined through a simple titration. A solution of the drug product in water is prepared at the same solution concentration intended for final use. The pH of the solution is adjusted to approximately pH 3 with concentrated hydrochloric acid (HCL) and a pH probe is placed into the solution. As known amounts of a sodium hydroxide solution are added using a burette, the pH of the solution is recorded. The following equation can then be used to calculate the buffer capacity at any pH:

$$\beta = \frac{dW}{dpH}$$

β = buffer capacity

dW = change in the number of moles of weak base added

dpH = change in pH based on added base

Figure 13-10 illustrates a typical pH vs. added base curve for a buffer system. Note that the region where the solution pH and pKa of the buffer are close (buffering region), the solution pH changes very little by the addition of added base. However, when the buffer is exhausted after adding approximately 15 mL of base solution, the pH rises dramatically.
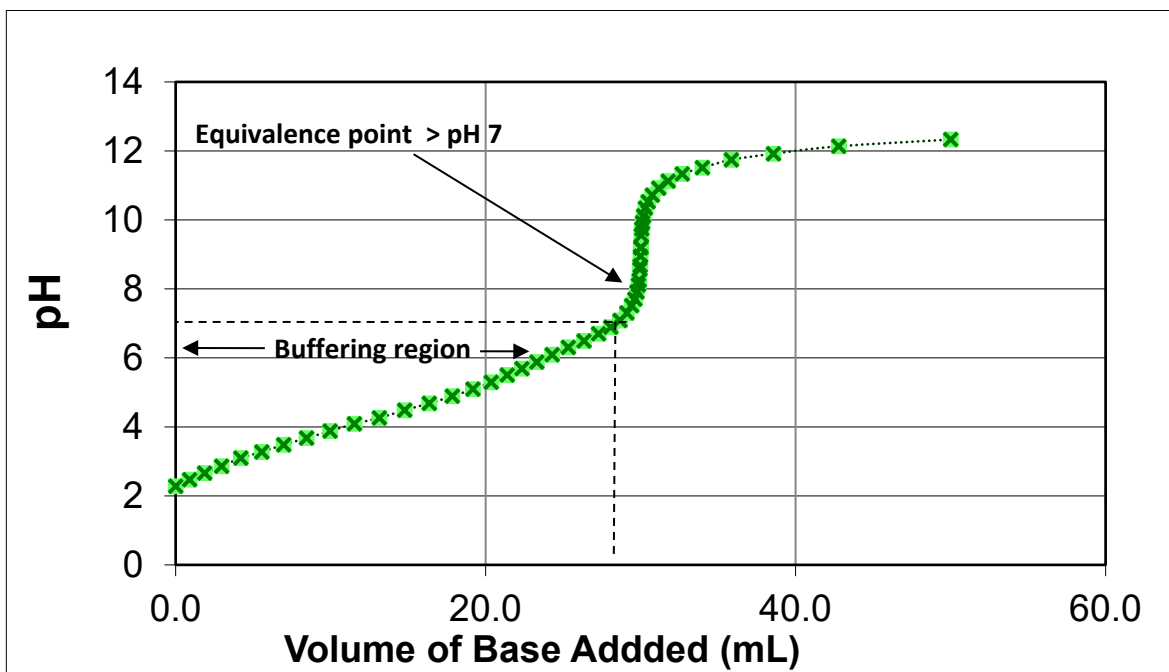

Figure 13-10. pH titration curve

Another useful equation for developing buffer systems is Van Slyke's Equation:

$$\beta = (2.303)(C_T)\frac{(K_a)(H^+)}{(K_a + H^+)^2}$$

β = buffer capacity
C_T = buffer concentration (M)
K_a = acid dissociation constant (-inv log of pKa)
H^+ = hydrogen ion concentration (-inv log of pH)

The pH and pKa are known from the studies described above, so this equation can be used to find the optimal buffer concentration for a desired buffer capacity and vice versa.

Antioxidants, stabilizers, surfactants, and bulking agents

Occasionally additional steps must be taken to help stabilize unstable products. If the pre-formulation studies were performed correctly, the information regarding the damaging effects of factors such as light, temperature, water, and oxygen should all be known prior to starting the formulation development studies.

If a drug is shown to be susceptible to damage from oxygen, there are several approaches that can be taken to protect it. The first approach is to rid the environment surrounding the drug molecule of the oxygen. Water holds a significant amount of oxygen (fish would die without it), all of which can interact with the drug and cause degradation. Before adding the drug to the solution, the liquid can be sparged with nitrogen to remove the oxygen from solution. Like a pH probe, which is sensitive to and can quantify the amount of hydrogen, probes that are sensitive to and can quantify the amount of oxygen are also available. By sparging the solution with nitrogen and monitoring the level with an oxygen sensitive probe, the dissolved oxygen can be reduced down to less than 1 ppm. Once the solution is sparged down, a blanket of nitrogen must be kept over the top of the solution throughout the remaining manufacturing and filling process to prevent the re-uptake of oxygen by the solution. During filling, manufacturers typically blow nitrogen into the empty vial, fill the drug, blow another burst of nitrogen over the liquid, and then seal the vial with a stopper. This is generally enough to protect the product from degradation due to oxygen. If this is not enough, excipients known as antioxidants can be added. Below is a partial list of acceptable antioxidants:

- sodium bisulfite
- sodium metabisulfite
- ascorbate
- sodium sulfite
- thioglycerol

These antioxidants should only be added if oxidation protection cannot be achieved through removing oxygen as described above, as these substances are irritating and cannot be used at high levels.

Other ways in which to control damage from oxidation are:

- avoiding high pH

- avoiding high temperature

- avoiding added heavy metals

- avoiding peroxides

- avoiding long exposure to light

Stabilizers are used to help molecules such as large proteins keep their folded structure, especially in response to freezing and drying stresses associated with freeze-drying. The molecules that have been shown to be good stabilizers are disaccharides such as sucrose or trehalose. These molecules essentially surround the protein and stabilize the folded structure thermodynamically via entropy and hydrogen bonding with the protein exterior.

Surfactants were described earlier in regards to acting as emulsifiers for stabilizing emulsions. These surfactants can also be useful for stabilizing formulations containing large protein molecules such as monoclonal antibodies. At times these protein molecules unfold in solution and their hydrophobic residues are exposed, which then stick together and form protein aggregates. Also, some proteins are attracted to different surfaces encountered during manufacturing, including glass, stainless steel, and process tubing. The surfactant essentially sticks to these surfaces preferentially over the protein, thus blocking the protein from getting stuck. The use of these types of molecules is limited, as they are irritating to the body. The most widely used surfactants for injectable drug products are the non-ionic polysorbate molecules, including:

- Tween 20

- Tween 80

Bulking agents are used specifically for products to be freeze-dried (this process is described in detail in another chapter). At times only a small amount of drug is required to treat a disease, and when freeze-dried, this small amount of drug is not visible, and the vial essentially looks empty. By adding a bulking agent, such as mannitol, glycine, or Dextran, the vial then contains an intact cake, thus there is known product present. While this is largely a cosmetic issue, it does ensure that there is product in the vial, and the bulking agent can at times help hold on to the product and keep it in the vials. For example, if only a small amount of wispy solids are left after freeze-drying, the bulking agent can hold on to them and keep them from being blown out of the vial (which would result in a subpotent dose).

Accelerated Stability Studies

During both the pre-formulation and formulation development studies, accelerated stability studies are conducted to determine the effects that different factors, such as excipients and processing, have on both the short- and long-term stability of the drug product. Typically formulations are prepared with variations, including pH, stabilizer, buffer, surfactant, and antioxidant, and placed at various temperatures to determine how quickly and by what means they degrade. The initial stability studies are performed at accelerated temperatures to speed up the time that samples degrade, so information can be acquired quickly to make changes to the formulation based on the results. At the end of a good pre-formulation and formulation development study, the development scientist should have three to five candidate formulations

to carry into a full scale development stability study. The results of this study are used to determine the final formulation that will be filed in the New Drug Application (NDA) document and forwarded to the Food and Drug Administration (FDA) for review. This is also the formulation that will be formulated and administered into people for the Phase III Clinical Trials, which if successful, will be the formulation of the product that will be marketed and administered to the general public.

The candidate formulations are prepared and placed at several different temperatures. Generally samples are placed at -40°C (as controls), 5°C, 25°C, and 40°C. Depending on the thermal stability of the drug, some companies may place samples at 50°C as well.

Drugs in solution generally degrade according to what is known as first order kinetics, which means that the rate at which they degrade depends on the amount of drug left at that time. This means that the drug degrades exponentially, much like radioactive decay. Samples placed at the different temperatures are tested over time for the amount of drug that is left, and from this a rate of exponential degradation, or rate constant (k), can be determined. Since the rate of degradation is different depending on the storage temperature, a different rate constant can be calculated for each temperature. By plotting the log of the concentration lost vs. time (Figure 13-11), the rate constant can be determined by using the equation below.
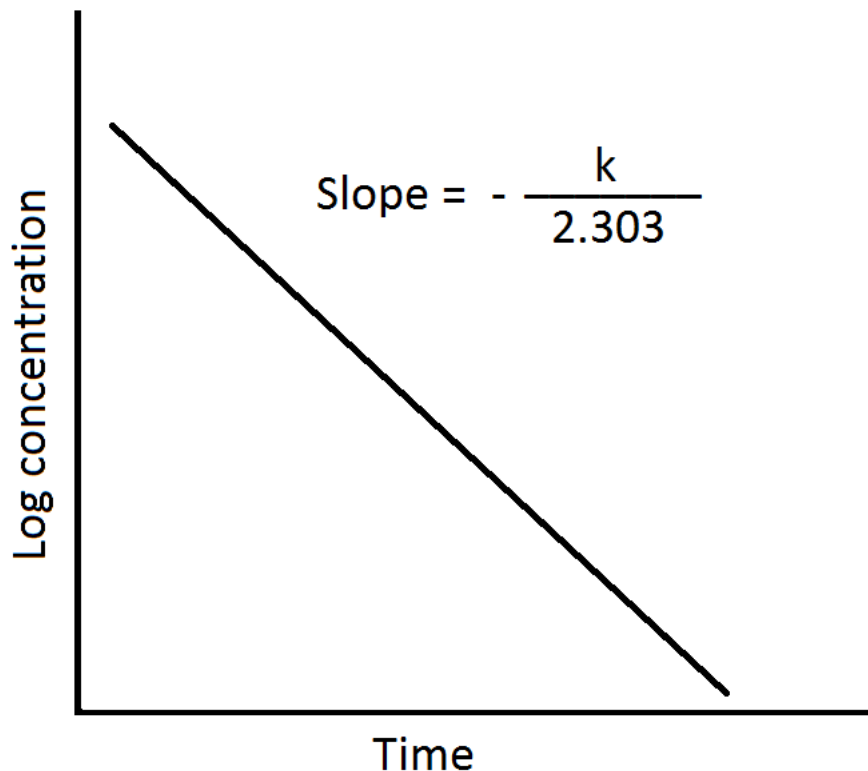

$$Slope = -\frac{k}{2.303}$$

Figure 13-11. First order rate constant curve

$$log\ c = log\ c_0 - \frac{kt}{2.303}$$

c = the drug concentration at time t
c_0 = the initial drug concentration
k = the rate constant
t = time

The above equation is an equation of a line, which means that the slope of the line equals -k/2.303, and the y-intercept = log c_0. Once the rate constants have been determined at each temperature, an Arrhenius plot can be made using the following equation:

$$log\ k = log\ A - \frac{E_a}{(2.303)(R)(T)}$$

E_a = energy of activation
k = rate constant
R = gas constant
T = temperature

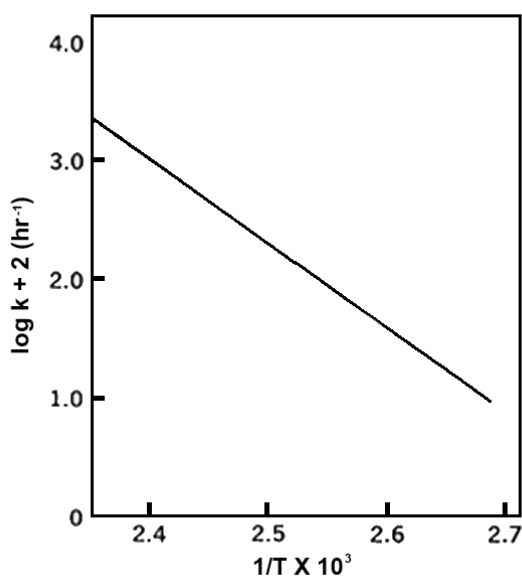When graphed as in Figure 13-12, the slope of the line = $-E_a$/2.303R, and the y intercept = log A.

Figure 13-12. Arrhenius plot graph

Once the rate constants are determined from the accelerated stability studies, the shelf life of the drug can be determined at any temperature and over any length of time. The temperature can be entered into the equation above to get the rate constant (k). Once the rate constant is determined at a certain temperature, it can be used in the equation below to determine the shelf life of the drug at any time and temperature.

$$C_t = C_0 e^{-kt}$$

C_t = drug concentration at time t
C_0 = initial drug concentration
k = rate constant
t = time

Process compatibility and scale-up

Once the final formulation has been developed, transferring the formulation, and requirements for filling and freeze-drying, to a manufacturing facility must be performed to ensure that the product can be made flawlessly while meeting the same release specifications that were established during the development studies.

Product Hold Time Study

If issues with stability were found in the pre-formulation studies, then a hold time study should be conducted. The drug product is formulated, tested for potency/purity, held at a fixed temperature, and then tested over a 24-hour period, generally pulling samples at 2, 4, 6, 8, 12, and 24 hours. If the product is extremely sensitive to degradation, the study can be performed under refrigerated conditions (5°C) as well. It is not uncommon for highly sensitive products to be kept cold during formulation and filling.

Process Compatibility Study

Prior to preparing a batch of the drug product at a manufacturing facility, a process compatibility study should be completed. The drug product comes into contact with several different components during the manufacturing process, some of which may have a negative interaction with the product. A sample of the drug product is formulated, tested for potency purity, and then exposed to the following:

- glass

- stainless steel

- process tubing

- plastics

- other components that may come into contact with the drug product

As in the product hold time study, samples are pulled at regular intervals (2, 4, 6, 8, 12, and 24 hours) and tested for potency and purity.

Filter Studies

Finished injectable drug products must be sterile filtered (unless a suspension) using a filter with a pore size smaller than the size of typical microorganisms (0.22μm). (There are several different types of filter membrane materials (discussed in a separate chapter). The type of filter material chosen depends upon the types of formulations to be filtered. For a typical, water-based formulation of a small molecule, a typical filter membrane would be a polyvinylidene fluoride (PVDF) membrane.

Two studies in particular should be conducted when determining if a specific filter membrane is compatible with a formulated product—a filter compatibility study and a flow decay study. The filter compatibility study ensures that the drug product passes through the filter without having

drug loss due to binding of the drug to the membrane. This is typically performed using a syringe filter containing the same membrane that would be used in manufacturing the drug product. Typical syringe filters are depicted in Figure 13-13.

Figure 13-13. Syringe filters
(courtesy Krackler)

A sample of product is formulated and tested for potency, and then samples are collected after various volumes of the product have passed through the filter. These samples are tested for potency to ensure that it is not lost when passing through the filter. Typically samples are collected and tested after 0.5, 1, 2, 5, and 10 mL.

The second study to be conducted is a flow decay study. This study ensures that the proper sizes of filters (and potentially a pre-filter) are used so they do not become plugged during the sterile filtering process; most filter vendors offer this service free of charge. A sample of the product is formulated using the same excipients and active pharmaceutical ingredient that will be going into the product. The weight of the product filtered is then recorded over time as it goes through the filter. Using a calculation based both on the results and the estimated volume to be produced by manufacturing, both the appropriate filter surface area and the need for a pre-filter are determined.

Scale-Up

Once the formulation is developed; the process compatibility studies are complete; and the batch record has been written and approved, the product is ready to be scaled up from a lab batch to a large batch prepared in production. This can be either a fairly simple process for small-molecule, water-based formulations or a relatively complex process for batches such as large proteins, emulsions, or freeze-dried products. Producing a scale-up batch is basically a practice run to ensure that product made in production meets the same specifications as the material made in development without any mechanical glitches occurring during the process. For expensive drug products, scale-up studies can be conducted using a suitable placebo. If the drug product passes all of the standard QC release tests, and there were no major mechanical issues during the process, the product can then be produced at a large scale in manufacturing.

Check Your Knowledge

1. Which of the following is not a disadvantage of using an injectable drug product?
   a. pain on injection
   b. bypasses the body's natural defenses against infection
   c. drug may be unable to pass through a needle
   d. site needs to be sterilized before administration

2. Which of the following is not a type of injectable product?
   a. solutions ready for transfer
   b. dry, soluble products (freeze-dried or powder fill) ready to be combined with a solvent just prior to administration
   c. suspensions ready for injection
   d. dry, insoluble products ready to be combined with a solvent prior to administration
   e. emulsions
   f. liquid concentrates ready for dilution prior to administration

3. According to the Stokes Law approach for suspensions, the suspending effect is due to what?
   a. altering the repulsive charge on the particles
   b. making the particles larger
   c. slowing the velocity of the settling particles
   d. making the particles smaller

4. When oil and water are added together to make an emulsion, what should be added to prevent the oil from separating and floating to the surface?
   a. a suspending agent
   b. a bonding agent
   c. a lipopolysaccharide
   d. a surfactant

5. What is one of the first items required before starting any pre-formulation or formulation development work?
   a. the solubility profile
   b. the pH stability profile
   c. a stability indicating analytical method
   d. a complexing agent

6. Which of the following refers to the strength of a buffer system?
   a. buffer strength
   b. buffer capacity
   c. buffer capability
   d. none of the above

7. The pH where the buffer has equal part acid and base components is called what?
   a. isoelectric point (pKI)
   b. depletion constant (DkA)

c. acid dissociation constant (pKa)

d. deactivation constant (Kda)

8. Which of the following is the simplest way to remove dissolved oxygen from a solution?

a. chemically interact oxygen with scavengers

b. add antioxidants

c. sparge with inert gas while mixing

d. keep it protected from light

9. Drug products in solution generally degrade under which order of kinetics?

a. zero order

b. first order

c. second order

d. third order

10. A filter study is conducted to determine if it will contaminate a product with endotoxin?

a. true

b. false