fully inserted. Completing the insertion creates a slight overpressure in the headspace resulting in a tendency for stoppers to "pop up" slightly after insertion. To address this, a "no-pop" ring can be molded into the stopper plug and a corresponding "blowback" ring can be formed into the neck of the vial. The intention is to provide additional mechanical interference to help retain the stopper in the seated position until the aluminum overseal is positioned and crimped. Here also, care is needed to ensure that the design details of each component are appropriately sized and positioned. The container system designer is advised to work closely with the component manufacturers to ensure compatibility.

The blowback feature originally was developed for smaller containers, for example, a vial with a nominal fill capacity of 2 cm^3 having a fill volume of 2 mL plus overage. In this situation, the volume of the stopper plug can be a significant percentage of the total headspace volume which increases the likelihood of pop-out because of pressurizing the headspace. Pharmaceutical companies producing lyophilized products also recognized the possibility for the blowback feature to improve the control over the position of the partially inserted stoppers during transfer of filled vials between the filling suite and the lyo chamber. Thus, vials and stoppers for lyophilization also often incorporate blowback rings.

Prefilled Cartridges

Glass cartridges are tubular glass containers that are open on one end to receive a suitable elastomeric plunger stopper. The opposite end has been tooled to form a neck and flange. After filling, the tooled end is closed with an aluminum cap which is lined with a suitable elastomeric septum. Just before use, a double-ended needle is attached. When the needle is attached, the end of the needle at the aluminum seal pierces the septum allowing the medication to be administered. Dental anesthetics and insulin therapy are two important markets for prefilled cartridge systems. For ease of use, the systems often are combined with reusable holders or, increasingly, adjustable multidose pen devices. Compared with a vial of equal capacity, a cartridge-based system will be longer, smaller in diameter and have little or no headspace gas. ISO has defined materials, dimensions, performance, and test methods for the product contact components of such systems in ISO 11040. Parts 1 and 4 (16,17) of the standard are glass cylinders, while parts 2, 3, and 5 address plungers, septa (disks) and aluminum caps. Additional requirements for components used in pen-injector systems are defined in ISO 13926 (18) parts 1 through 3.

The glass forming process for the finish of a pen cartridge is similar to that used to form the neck and flange of a tubular vial. Online 100% inspection and off-line quality control checks also are similar. Cartridges are produced from tubing and can be formed using either one of two basic process concepts. The neck and flange may be formed, as with tubular vials, on the end of the tube. After forming the finish, the cartridge is separated from the tube using thermal shock and the open end is flame polished. Alternatively, full length tubes may be first cut into blanks using thermal shock and flame polished. On a separate forming line, the flange and neck are formed on one end of each blank. The smoothness and uniformity of the open end can have an important effect on the ability of the finished cartridge to endure the rigors of packaging and distribution.

In addition to its role as a drug product container during shelf life, at the time of use, the cartridge also plays a functional role as part of the drug delivery system. To fulfill this function, the body of the cartridge must be lubricated to reduce and control the static and dynamic friction between the glass cylinder and the elastomeric plunger. Generally, the lubricant is an emulsion of polydimethylsiloxane that is added to the final WFI rinse prior to depyrogenation using dry heat. The depyrogenation process drives off the residual water leaving behind the lubricating silicone layer. The interaction between the glass surface, the silicone fluid, the drug product and the elastomer plunger is complex. The processes affecting this interaction should be characterized thoroughly, validated and monitored to ensure consistent functional performance throughout shelf life. This is especially important for pen-injector systems where precise dosing is required. Cartridges for injection devices also may have additional dimensional requirements related to dose accuracy or to fit and function within the device.

Prefilled Syringes

In some ways, prefilled syringes can be considered an extension of the cartridge concept. Prefilled syringes also are formed from glass tubing. With a cartridge, one end is open to receive a suitable elastomeric plunger stopper. Unlike cartridges, the open end of a prefilled syringe is tooled to form a finger flange by which the syringe is held during administration of the dose. The opposite end of the syringe may be tooled to the shape of a male luer taper or to accept a plastic luer lok adapter or a small channel may be formed at the inner diameter of the tip into which a cannula is later inserted and glued. In each case, prior to filling, the syringe tip is fitted with a suitable elastomeric luer tip cap or needle shield. Prefillable syringes can be supplied as "bulk" (unprocessed) containers intended to be rinsed, siliconized and sterilized just prior to filling. Luer tip and Luer Lock syringe barrels can tolerate dry heat depyrogenation and the tip cap or tip cap and adapter are assembled under aseptic conditions in the filling suite. The adhesives typically used on syringes with glued in cannulae cannot tolerate dry heat. "Bulk" staked needle syringes are sterilized by autoclaving rather than by dry heat.

As with cartridges, prefilled syringes are produced from tubing and can be formed using either one of two basic process concepts. The tip may be formed, as with tubular vials, on the end of the tube. After forming the tip, the syringe body is separated from the tube using thermal shock and the open end is flared and tooled to form the finger flange. Alternatively, full length tubes may be first cut into blanks using thermal shock and flame polished. On a separate forming line, the finger flange is formed on one end of each blank and the tip is formed on the other end. The flange forming process may occasionally reduce the inner diameter at the flange opening. This may affect processing when mechanical plunger setting tubes are used.

Numerous dimensional and functional attributes of the glass barrels and various in-process assembly steps for prefilled syringes are 100% inspected using camera-based systems. Other process control and quality checks are performed at the appropriate stages of production using both time-based and AQL-based sampling plans.

In addition to bulk, unprocessed syringe barrels, there also is a significant and growing market for prefillable syringes that have been rinsed, siliconized, suitably packaged and then sterilized by the syringe manufacturer. These ready to fill systems are sterilized by ethylene oxide using validated cycles. Sterility testing is routinely performed on each sterilization batch.

As with pen cartridges, prefilled syringes serve double duty as the container-closure system during shelf storage of the drug product and as an integral part of the drug delivery system at the time of use. In prefillable syringes, the lubricant generally is applied as an aerosol mist of silicone fluid. The processes affecting this aspect of the syringe system should be well understood and controlled to ensure consistent functional performance.

For prefilled syringes, there is an additional level of complexity in that the tip cap or needle shield also serves a dual purpose. During shelf storage, this product contact interface is an integral part of the container-closure system. Yet, at the time of use, the tip cap or needle shield must be easily removed. And, for a luer tip or luer lok syringe, system performance requirements include the ability to form a leak-tight seal with the injection needle or delivery system adapter. Prefilled syringes also are increasingly being incorporated into automatic injection devices. Additional specification requirements and quality control tests may be required to ensure consistent drug delivery performance of prefilled syringes and auto-injectors.

While the focus of this chapter is on glass containers for parenterals, it is important to recognize that from the perspective of drug product compatibility, prefilled cartridges and prefilled syringes have added complexity compared with vial-stopper-seal systems. At a minimum, these systems include a second elastomer in the septum, tip cap or needle shield in addition to the plunger stopper. These systems also include the silicone fluid lubricant on the barrel and generally on the plunger stopper as well. Finally, for syringes with preattached needles, the stainless steel cannula and adhesive are in direct contact with the drug product throughout shelf life. The potential effects of each of these additional product contact materials needs to be assessed during qualification of the container-closure system.

Specialty Items

Other special purpose container systems, such as dual chamber vials, cartridges and syringes, threaded vials for infusion systems and high-strength capsules for needle-free injection systems also are available. An exhaustive review of these systems is beyond the scope of this chapter. The interested reader is encouraged to contact glass container manufacturers to learn about speciality products and new developments.

SURFACE CHEMISTRY

There are two fundamental mechanisms of chemical attack that can occur when an aqueous solution is in contact with the surface of a glass container (19). Through ion exchange, H_3O^+ ions in the solution can replace Na^+ ions in the glass. Once the sodium ions have been removed from the near surface layer, the rate of diffusion of sodium ions from within the bulk glass slows the process considerably. Ion exchange is the dominant mechanism of attack for most acidic and neutral formulations.

By contrast, hydroxyls and other alkaline species attack the silica network itself by breaking Si-O bonds. The rate of attack is highly dependent on the glass formulation and the solution pH. Surprisingly, several investigators (20 23) have shown that, at the same pH, different buffer systems can have markedly different rates of attack. It has been speculated that chelating agents are more aggressive toward glass because they are able to pull the various metal ions out of the surface. The resulting voids are then more susceptible to the other mechanisms of attack. Unfortunately, this means that simple formulation guidelines based on pH alone are not adequate.

In addition, the chemical resistance of the container surface also may vary. As mentioned earlier, the forming process can alter the composition, morphology and physicochemical characteristics of the container surface. During forming, especially when making the bottoms of ampoules and tubular vials, the temperature of the inner surface can exceed the boiling point of the more volatile ingredients of the formulation, primarily sodium and boron. These elements can vaporize from the hotter surface of the bottom and subsequently condense on the cooler sidewall as sodium borate. Then, as the finished container passes through the annealing oven, the deposits can be partially reintegrated into the underlying silica network. As a result, the alkaline deposits may not be completely removed by the pharmaceutical company's rinsing process but remain as less durable regions of the surface that is in contact with the drug product. This phenomenon will occur to some extent in the production of any container from glass tubing. For molded borosilicate glass bottles, vaporization and condensation of alkaline ingredients is generally not significant since the peak temperature of the glass is inherently lower. The resulting quantity of alkaline residue can be controlled by production speed, heating rate and maximum glass temperature. Residual alkalinity can be monitored by testing the surface resistance of the finished containers.

The alkaline residues can affect the drug product through three separate but related mechanisms. Firstly, the locally alkaline region or leached ions may react directly with the formulation. Secondly, by ion exchange with Na^+ ions in the glass, the loss of H_3O^+ ions from the solution can increase the pH of unbuffered or weakly buffered solutions. Thirdly, in extreme cases, the interaction can trigger the formation of an unstable layer of silica gel which can slough off as delaminated glassy particles.

Chemical dealkalization of borosilicate containers, for example, by the introduction of ammonium sulfate solution into the containers just before annealing, has been used, especially in the United States, as a means to control or minimize these effects. This process has been shown to be highly effective in reducing extractable alkali and the related effect on pH. Some users have found that the combination of controlled alkalinity in the forming process plus chemical dealkalization yields precise pH control for unbuffered products. However, studies by Ennis (24) showed that ammonium sulfate treatment without proper forming process controls did not eliminate delamination. In fact, in those studies, higher quantities and concentrations of treatment solution increased the formation of glass flakes.

Unpublished studies with which the author is familiar showed that delamination resulted from an interaction between excessive residual alkali on the vial surface, the parameters of the rinsing and depyrogenation processes, and the pH and composition of the drug product vehicle. Anecdotally, acidic residues from excessive dealkalization also have been reported to have caused a reduction in drug product pH and long term damage to washers and deypryogenation tunnels.

Phenomena such as these highlight the importance of evaluating the chemical durability of the inner surface of the finished container using, for example, the USP Surface Test, the Ph. Eur. test for surface hydrolytic resistance, ISO 4802-1 (25) or similar quantitative spectroscopic surface extraction test methods such as ISO 4802-2 (26).

MECHANICAL AND THERMAL PROPERTIES

The preceding section addressed the chemical properties of the product contact surface, which can be of vital importance to the physical and chemical stability of drug products stored in the containers. Physical integrity of the container as a means to maintain product sterility is another equally important requirement of containers for parenterals. In this respect, the mechanical and thermal characteristics of glasses must be considered. Earlier in this chapter, glasses were described as amorphous materials exhibiting the stress-strain characteristics of a brittle, elastic solid. Describing glass as a "brittle" material is perhaps consistent with the general perception that glass is fragile. By contrast, the notion that glass is "elastic" seems contradictory. However, as material science terms, brittle and elastic have more precise meanings both of which apply to glasses.

In this context, brittle refers not to the strength of the material but to the failure mode when local stress exceeds local strength. Most metals, when overloaded, will deform in a permanent way, technically, "plastic deformation," before breaking. Brittle materials, such as glasses, are unable to undergo plastic deformation and therefore break abruptly (27). Intrinsically, glasses are very strong materials in response to compressive loads. However, surface damage significantly reduces the effective strength under tensile stress. A compressive load squeezes the margins of a surface flaw or discontinuity together and has little effect. By contrast, a tensile load pulls a surface flaw or discontinuity apart and concentrates the stress at the bottom of the discontinuity. Thus, the flaw or discontinuity significantly reduces the practical strength of the material as elucidated by Griffith (28).

Similarly, as a material science term, elastic refers to the response of a material to the application and removal of a mechanical load that does not exceed the strength of the material. Elastic materials deform when loaded then return to the original shape when the load is removed. The stiffness of a material can be characterized by its elastic modulus, also known as Young's modulus, which is the ratio between the applied unit load, or stress, and the resulting unit deformation, or strain. In this respect, glasses are relatively stiff. Typically, the elastic modulus of glass is about the same as aluminum (29). Jiang (30,31) attached strain gages to the outer surface of glass vials to observe in real time the physical deformations of and corresponding stresses in the vials during freezing, frozen storage and subsequent rewarming and thawing of various buffers and formulated drug products. Although it was not the objective of the studies, the work demonstrates the elastic deformation of the glass in response to the changing physical dimensions of the contents.

Because of the combination of stiffness, brittle behavior and reduction in strength at surface flaws, one does not usually observe directly the elastic deformation that occurs in glass containers before catastrophic brittle failure occurs. Indirectly, when failure occurs, the energy stored by elastic deformation may be observed in the form of rapid fracture propagation and dispersion of the glass fragments.

Stress in glass containers can result from forces exerted on the container, either externally or internally. Stress also can be the indirect result of nonhomogeneous composition or other imperfections from the melting process or from thermal effects. Thermally induced stresses may be either permanent artifacts from the glass forming process or a transient response to temperature gradients within the glass. Moreover, stress in the glass is additive. The total stress at a given point is the sum of the stresses at that point regardless of the source.

Silicate glasses have relatively low thermal conductivity. As a consequence, heating or cooling results in a steep temperature gradient between the heated or cooled surface and the underlying glass core. This is the reason that the coefficient of thermal expansion of the glass composition is important in determining the thermal resistance of a container. When a container is cooled, the outer surface tries to contract. The contraction at the surface is resisted by the warmer core resulting in tensile stress at the outer surface. While this phenomenon is the principle behind "cutting" glass by thermal shock, it also can lead to unintended cracks during container production as well as during pharmaceutical processing.

For a given temperature difference, the stress level is proportional to the thermal expansion coefficient and the modulus of elasticity of the glass composition (32). Thus, all other conditions being equal, a 33-expansion borosilicate glass container can withstand a temperature difference on the order of three times larger than a container of identical size, shape and geometry made from a "90-expansion" soda-lime glass. It should be noted that, in addition to the properties of the glass, the cooling rate, the geometry of the container and the presence of surface flaws caused by handling all contribute to thermal resistance.

QUALITY ATTRIBUTES

Several aspects of quality control already have been mentioned in the discussions of the manufacturing processes. These described the process points where quality control checks are performed rather than the quality attributes being examined. A detailed discussion of the full range of possible container defects and cosmetic flaws is beyond the scope of this chapter. Nevertheless, it is worthwhile to point out that certain types of flaws can occur only in specific process steps. As such, some basic knowledge can be helpful when investigating container defects and failures. For example, glass flaws known as knots, stones, cord, seeds, blisters and airlines all originate in primary glass melting and tubing manufacture. Certain types of surface blemishes can occur only during blow-molding or conversion of tubing into ampoules, vials, cartridges or syringes. Finally, there are blemishes and defects that are more likely to be the result of interactions between containers and fill-finish equipment or processes. On the other hand, scratches, scuffs, bruises, and metal marks may occur at any process or handling step. Even in these cases, though, detailed examination may yield clues pointing to the root cause. For example, a scratch running the full length of the body of a tubing vial and fading into the heel and shoulder may indicate that the scratch was present on the tube prior to forming the container. Similarly, the location and orientation of a scuff or metal mark may eliminate most potential points of contact. The interested reader is advised to explore these topics with container producers. In addition, the Parenteral Drug Association (PDA) has published lexicons of attributes for tubular vials and molded bottles (33). Similar lexicons are being developed for ampoules, cartridges and prefilled syringes.

In some situations, the use of more sophisticated analytical tools may be warranted. Glass fracture analysis is the science of determining the origin of the breakage and the nature, direction and relative magnitude of the force that caused the breakage. Scanning electron microscopy with X-ray diffraction analysis or similar methods can be used to determine the elemental composition of surface flaws or of foreign materials that may be present.

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12 Plastic packaging for parenteral drug delivery

Vinod D. Vilivalam and Frances L. DeGrazio

INTRODUCTION

Driven by the development of biotechnology products, newer drug therapies, and reformulation of poorly soluble drugs, parenteral delivery is expected to provide strong growth in years to come. Routes of administration include subcutaneous, intramuscular, intradermal and intravenous injections. Drug products have been almost exclusively dispensed in glass containers, primarily because of the clarity, inertness, barrier property and thermal resistance of these containers. With the development of plastic polymer technology over the last 30 years, plastics have become logical alternatives for small-volume parenteral (SVP) and large-volume parenteral (LVP) packaging. Although plastic containers have become well-established as containers for LVP products, plastics have been, until recently, used on a limited scale for SVPs.

Glass vials are the primary container of choice because of their excellent gas and moisture barrier properties. More importantly, there is an extensive knowledge base on processing, filling, regulatory review and commercial availability of glass containers. Glass, however, may not be the best solution for all chemical or biological drug candidates. Glass contains free alkali oxides and traces of metals. Depending on the characteristics of the drug being packaged, it is likely that delamination could occur for high pH products over time, thereby affecting the shelf-life of the drug product. Proteins and peptides can be readily adsorbed onto the glass surface and can be denatured or become unavailable for treatment. With a glass prefillable syringe (PFS), potential leachables such as silicone, tungsten and adhesive can affect the stability of biopharmaceutical products. Glass may break during processing or transportation and when stored at low frozen temperatures. In these and other areas, plastic containers have made clear in-roads in the parenteral drug delivery market.

With the proliferation of new polymers and newer process technologies, most of the less-desirable characteristics of plastic containers have been overcome and the use of plastic packaging as vials and syringes is increasing. This chapter will discuss the role of plastic in pharmaceutical parenteral drug delivery. The discussion will provide insights on the following areas:

- Advances in plastic resins for SVP packaging with an emphasis on cyclic olefins as well as other plastics used: The properties of these plastics, applications and challenges will also be discussed.
- Plastic vial systems: This section will discuss in detail the development activities in this area including the use of plastic vials in lyophilization and the use of reconstitution devices.
- Plastic PFS systems: As more biopharmaceutical drugs and higher viscosity formulations are delivered in a PFS, there is the need for a break-resistant, high-quality, plastic PFS. Challenges with glass include breakage, reactivity of glass and leachables, such as silicone, tungsten and adhesive. Discussion will include how plastic PFS offer options to solve these challenges.
- IV bags and disposable bags: Following a brief overview of use of plastics for IV bags for LVPs, discussion will focus on new developments in the use of plastics for disposable bags in the packaging of biologics, including considerations for selection of disposable bags.
- Quality and regulatory considerations: U.S. Pharmacopeia (USP), European Pharmacopoeia (Ph.Eur.) and Japanese Pharmacopoeia (JP) compendial requirements will be discussed and referenced for plastic containers.

This chapter provides the reader with adequate information on recent developments, availability and use of various plastic packaging systems for pharmaceutical drug products, including suitable references to commercialized drugs products.

ADVANCES IN PLASTICS

Plastic resins are the most widely used raw materials in global pharmaceutical packaging, accounting for 61% of consumption compared with glass, paper products and aluminum foil. The worldwide demand for plastics for packaging was estimated at $25.8 billion or 2.3 billion lbs. of material consumed in 2006 (1). High-density polyethylene (PE) is the most widely used plastic with 1.2 billion lbs. consumed, followed by polypropylene (PP) at 0.4 billion lbs. However, the fastest growth is expected with the newer resins, the cyclic olefins growing at a compound annual growth rate of 5.5% by 2011 (Fig. 1). The growth is expected to penetrate specialty fields such as pharmaceutical drug delivery. This is driven by a need for clear, highly transparent, biocompatible packaging systems with improved quality and improved barrier protection.

Cyclic olefins: Compared with the traditional plastic resins, the development and application of cyclic olefins in parenteral drug delivery is relatively new. Cyclic olefins are prepared by additional polymerization of monocyclic olefins, cyclobutane or cyclopentane or bicyclic olefins such as norbornene. The resulting product has improved chemical and physical properties, such as glass-like transparency, excellent chemical resistance and improved moisture barrier. Mitsui Petrochemical Industries produced copolymers of ethylene and other cyclic olefins. Starting in the 1980s, Mitsui and Hoechst (2,3) began using single-sited metallocene catalysis in the polymerization of cyclic olefins that led to the development of the cyclic olefin copolymer (COC) Topas® by Ticona. In this process, 2-norbornene was reacted with ethylene in the presence of a metallocene catalyst to produce a series of copolymers whose properties can be modified by varying the norbornene percentage in the material. Another commercially viable route is through a two-step process based on the ring-opening metathesis polymerization (ROMP) of dicyclopentadiene followed by complete hydrogenation of the double bonds to form cyclic olefin polymers (COP) (Fig. 2). Using this process, the Zeon Corporation developed the Zeonex® and Zeonor® line of COP. A similar process also resulted in another clear COP plastic, called Daikyo Crystal Zenith® (CZ) that is available only in a finished container format from Daikyo Seiko, Ltd.

COP and copolymers (COC) possess many excellent properties, including glass-like transparency. This glass-like transparency of the polymers permits visual inspection of

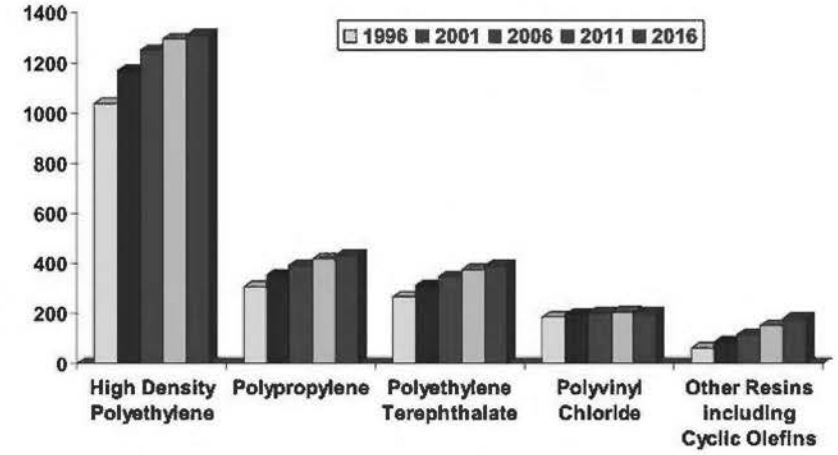


Figure 1 (*See color insert*) World pharmaceutical packaging plastics demand by resin (million pounds by weight).

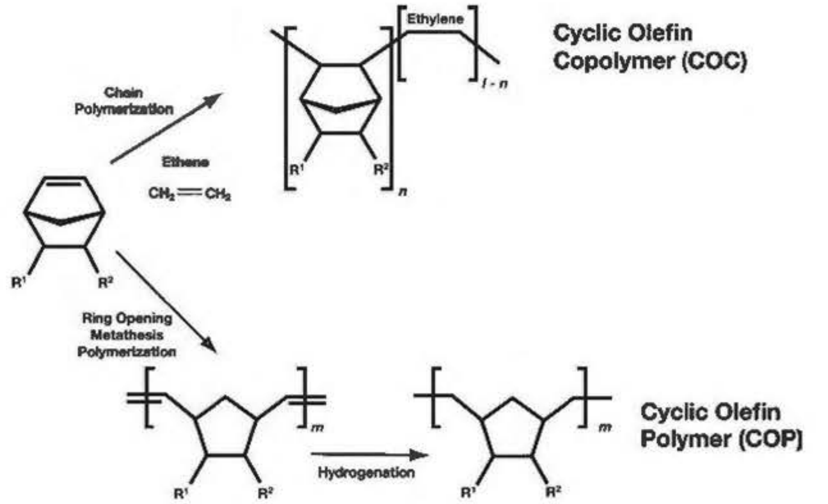

Ethylene
l - n
n
R¹
R²
Cyclic Olefin Copolymer (COC)
Chain Polymerization
Ethene
CH₂==CH₂
R¹
R²
Ring Opening Metathesis Polymerization
m
Hydrogenation
m
R¹
R²
R¹
R²
Cyclic Olefin Polymer (COP)

Figure 2 Process of polymerization in the development of cyclic olefin polymers/cyclic olefin copolymers. *Source*: Reproduced from Ref. 2.

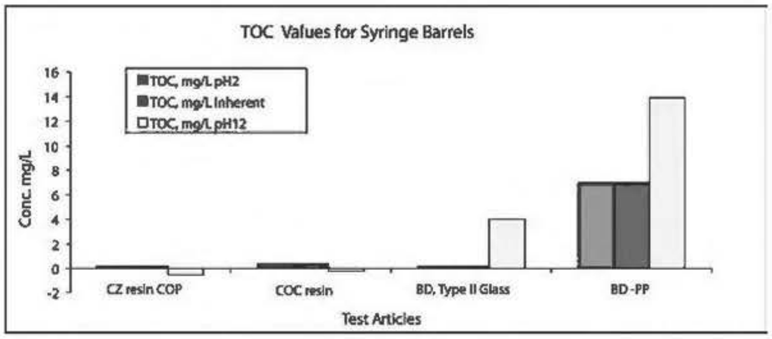


Figure 3 (*See color insert*) Comparison of total organic carbon as an extractable from syringe barrels. *Source*: Reproduced from Ref. 6.

the resultant manufactured components, as well as the parenteral products that are delivered to the end user. The polymers have good melt flow properties that readily lend themselves to plastics processing, for example, molding and thermal forming. The polymers exhibit a high impact and break resistance, and they form an excellent moisture barrier (2 5). Additionally, they possess good chemical resistance to acids, bases and alcohols. These polymers are sterilizable by autoclave, ethylene oxide and radiation sterilization processes. As with most plastics in comparison with glass, the number of potential compounds that may be an extractable or leachable is higher for plastic than for glass because the number of components in the formulation is higher. These compounds are organic, whereas glass potential extractables are inorganic. Plastic vendors can provide a list of potential extractables developed with suitable extracting solutions. A decision may then be made on which potential extractables should be studied as leachables during stability testing. Preliminary studies have shown that, when compared with other materials that are used for parenteral applications, COP and COC exhibit very low extractables (Fig. 3). When studied for total organic carbon (TOC) extracted from syringe barrels at various pH levels, the

Table 1 Features of Cyclic Olefins for Parenteral Drug Delivery

Key benefits	Drawbacks
Glass like transparency	Gas and moisture barrier properties are less than glass but better than other plastics
Sterilizable (via autoclave, radiation and ethylene oxide)	Sensitivity to scratches
High break resistance	Short term discoloration due to radiation
Excellent moisture barrier	
Biocompatible (inert, low binding, and ion extractables)	
Design flexibility and excellent dimensional tolerances	
Good chemical resistance	

data shows very low extractable for COP (CZ) and COC compared with PP and glass (6,7). On the basis of this data and other information available, COP and COC are considered to be ideal plastic packaging containers for SVP. There are some drawbacks, however. Understanding these drawbacks will be important in the selection of cyclic olefins as a packaging system (Table 1). These plastic containers cannot match the barrier properties of glass to oxygen and moisture ingress, although they are much superior to other plastics, including PP, polystyrene and polycarbonate (PC). For oxygen-sensitive compounds, this may be a concern. Suitable secondary packaging can prevent moisture loss or oxidation, with the addition of a moisture absorbent or oxygen scavenger material.

High density polyethylene (HDPE): The polymer is based on a simple repeating carbon/hydrogen molecule that branches out during polymerization to form a polymer with a high degree of regularity. This regularity creates the formation of crystal lattice structures. Polyethylene (PE) is recognized as having a high degree of crystallinity. During polymerization, the amount of branching that occurs during the process will determine the overall density and crystallinity of the resulting PE. As a result of their relatively high degree of crystallinity as compared with lower-density PEs, HDPEs have greater tensile strength and stiffness and have a higher melting point than the low-density polyethylene (LDPE) resins. Another important property is excellent chemical resistance, a characteristic of all polyethylene grades. HDPE is typically used for low-to-medium barrier medications, such as bottles, closures and in some cases, secondary packaging of parenterals and blister packs for solid dosage forms. The material is characterized by strong impact resistance, chemical resistance, drug compatibility for oral dosage forms and temperature tolerance. Both HDPE and LDPE are used to form containers by blow-fill-seal technology, primarily for ophthalmic and nasal/respiratory drugs, but also have been used for both SVP and LVP products.

Polypropylene (PP): PP is the leading plastic employed in containers, disposable syringes, PFS and closures. PP is a linear, high crystalline polymer, made of carbon and hydrogen in a very orderly fashion. The regularity of its structure imparts the high degree of crystallinity found in most commercially available PP. Within the crystal array, the methyl groups impart stiffness to the polymer, making it different from its close relative, polyethylene. PP exhibits a high tensile strength, which is the ability to withstand forces tending to pull apart or distort the material, and is more rigid than HDPE. High tensile strength, in conjunction with a high melting point of 165°C, is particularly important for packaging drugs. Consequently, the material has the ability to withstand higher temperatures of autoclave sterilization for a limited number of cycles. PP is also resistant to chemical attack from organic solvents and strong acids and bases at room temperature. Because of the level of crystallinity present, it is not possible to achieve the optical clarity found with cyclic olefins: the crystal lattice sites tend to refract light, which imparts haze. The resin generates significant demand for the manufacture of blow molded bottles, pouches, laminates and plastic containers. Because of its improved moisture resistance and effective chemical resistance, PP is typically used in disposable containers or delivery systems. It may have poor impact resistance at lower temperatures and increased extractables, and its translucency limits its role in the storage of parenteral drug and biological products for long duration.

Polyethylene terephthalate (PET): PET is a high-quality thermoplastic polyester that offers good barrier protection, chemical resistance and processing properties. It is typically used in packaging drugs that may require barrier protection as in blister packs and blow molded containers. It is cost competitive with HDPE and PVC and is used in development of bottles and blister sheeting. PET is polyester that is a condensed polymer prepared from ethylene glycol (EG) and either terephthalic acid (TPA) or the dimethyl ester of terephthalic acid (DMT). The EG monomer is prepared using ethane as feedstock and the TPA is manufactured using paraxylene as feedstock. TPA can then be purified by reaction with methanol to form the DMT. PET can exist in an amorphous state, an oriented and partially crystalline state and a highly crystalline state. Because of its low glass transition temperature, PET cannot tolerate autoclave sterilization. The material does hold up well to gamma radiation, making it the preferred method for sterilization. Ethylene oxide sterilization is also acceptable with PET resins. PET film may potentially be used as a coextruded layer of LVP bags (replacing use of PVC resins).

Polycarbonate (PC): PC is known for its mechanical properties and higher clarity with poor barrier properties. PC-based polymers are aliphatic molecules and are synthesized in various forms. These aliphatic PCs become extremely soft in the 40°C to 60°C temperature range. Bisphenol A PC is extremely stable and virtually nondegradable under physiological conditions. PC can be processed readily, possesses high mechanical strength and is very shatter resistant. PCs are used extensively as bottles and containers for parenteral applications. PC resin contains repeating aromatic rings in its main chain structure. The material is a polyester of carbonic acid and is generally produced using an interfacial reaction between dihydric or polyhydric phenols and a suitable carbonate precursor such as dichlorocarbonate. Currently most PCs are produced with a reaction between bisphenol A and carbonyl chloride in an interfacial process. Other polyhydric phenols are sometimes used to form copolymers for special end uses. The material is well suited for the injection molding process. PC shows excellent creep resistance over a broad temperature range, enabling its use in applications previously open only to thermoset materials. There are, however, some areas where PC resins are inferior. PC materials have limited chemical and scratch resistance and a very high water transmission rate when compared with other plastics. The resin also has a tendency to yellow with light exposure and with exposure to radiation sterilization.

Polyvinyl chloride (PVC): Less popular in parenteral packaging, PVC is prepared by polymerizing a gas, vinyl chloride or monochloroethylene, in the presence of organic peroxides or inorganic persulfates as initiators. The length of the molecular chain and the structure of the side chains are altered by the temperature, pressure and the nature of the initiator. PVC's growth in pharmaceutical packaging is much slower compared with its peers because of environmental concerns. This includes the formation of dioxin when PVC is incinerated. Additionally, di(2-ethylhexyl) phthalate (DEHP) plasticizers are used in the production of many PVC materials. These types of phthalates, which are known to leach out of PVC containers, may have potential health risks. Growth has slowed in this area, which probably reflects preferences for better performing and safer plastics.

Multilayer plastics: Plastic bags commonly used for LVP generally consist of between three and five layers of plastic film consisting of two or more different resins. Similarly, plastic film used for blister packaging of tablets is also multilayered. The purpose is to produce a plastic film that combines the best properties of each film including good clarity, excellent flexibility and durability, which also is a strong barrier to water vapor transmission.

Plastics fabrication: There are many processes used to convert plastic resins from pellets into desired shapes or configurations. This is a brief description of the plastic molding processes. All plastic processes are similar in the use of three basic elements to convert the resin from a pellet to its processed shape.

1. *Heat*: excites the molecular structure to allow free movement of molecules
2. *Pressure*: forms the free-flowing polymer into a desired shape
3. *Time*: allows the transfer of heat into the plastic followed by time for removal of heat (cooling)

Extrusion of plastics: The process of extrusion involves melting a plastic and forcing it through a die under pressure to form a desired shape. There are several types of extrusion, depending on the die arrangement used to form the plastic. The three most widely used for parenteral packaging are flat-sheet extrusion, profile-tubing and blown-film extrusion. Flat-sheet extrusion is a versatile process, with the capability to produce sheet stock over a wide range of thicknesses from a wide range of resins. The process may also be used to produce coextruded sheeting where two or more different resins are brought together in the die manifold from two or more extruders. Flat-sheet extrusions can be used for blister packaging and form, fill and seal packaging. Clear grades of plastic that have a high degree of stiffness are generally preferred for extrusion processing. Another application for this process is the production of LVP containers.

Injection molding: Injection molding is a process used to convert resin from a melt into a molded shape using a mold pattern to form the part. Injection-molded products are replacing materials such as glass, metals and paper in many areas of parenteral drug packaging. The development of newer plastic resins, combined with improvements in the injection molding process, is setting the stage for these changes. For example, materials such as CZ resin have been used to develop larger containers such as the 1-L bottle by injection molding. Many of these newer resins are used for drug delivery systems that are replacing products traditionally made from glass. In this process, plastic resin is melted using the extrusion process and is injected into a mold where the resin is cooled enough to be removed in a solid state. Like the other plastic processes, heat, pressure and time are used in each of the steps to produce a molded product. Injection-molded items are finding many uses in parenteral drug packaging. The injection molding process is also used to produce components such as IV spikes and IV administration sets.

Blow molding: The blow molding process has grown rapidly over the past three decades. The two types of blow molding in use are extrusion blow molding and injection blow molding. A uniform tube of heated resin with one end closed is formed during the extrusion blow molding process and is moved into a mold where the two ends are pinched off, and the material is blown outward into the shape of the mold. The injection blow molding process is similar in concept except that is a two-step process. A preform is molded using a first-stage mold and the principles of injection molding. The form is then transferred into a second mold, and blown outward using pressurized air to form the container. Containers produced for health care applications, such as tablet bottles, are made primarily using the injection blow mold process. With small containers, this process is more cost effective than extrusion blow molding because it is capable of handling a large row of preforms at one time. Extrusion blow molding lends itself to larger containers where it becomes more economical and practical to eliminate the preform step. The blow molding process enhances the physical, chemical and barrier properties of certain materials, for example, PET, because it creates a high level of bi-axial orientation of the polymer. CZ, Zeonex and Topas resins also use the blow molding process to manufacture vials.

VIAL SYSTEMS

Market considerations: A vial is a SVP container with a stopper and a seal, intended to package liquid or a dry powder formulation for either single or multiple doses. Glass vials, typically made of type I glass, are most commonly used as vials for parenteral applications. Recently there is increased interest in the use of newer plastics, particularly the cyclic olefins, as parenteral vials as they provide clarity and inert surfaces for biopharmaceutical and biological applications. When combined with plastic's inherent break-resistant attribute and the need for biologics to be stored and transported at lower temperatures, the future of cyclic olefin based plastics appears bright. Cyclic olefin polymers (COP) and copolymers (COC) are considered to be an ideal plastic for vial systems because they have glass-like clarity and suitable physicochemical properties and the ability to be sterilized.

The vendors in this area may be divided into those that manufacture the COP and COC resins such as the Zeon Corporation and Topas Advanced Polymers and companies that convert the resin into parenteral containers such as Schott Forma Vitrum that offers a range of sizes of both syringes and vials made out of COC under the brand name Schott TopPac®. Daikyo Seiko, Ltd. of Japan has used a proprietary COP resin to produce a range of sizes of conical, flat-bottom vials and larger screw-top containers, under the brand name of Crystal Zenith. West Pharmaceutical Services, Inc. (West) partners with Daikyo to codevelop, market and sell sterile and nonsterile CZ vials. As a result of the anticipated growth, the suppliers of resins and products have made significant investments to their supply chain to maintain continuity of supply. Rexam offers a new generation of multilayered plastic vials called MLx that are being used as a container with improved barrier properties. The COC vials produced by Aseptic Technologies represent a newer approach to vial handling and filling called the Crystal® technology, licensed from Medical Instill Technologies (Table 2). The vials and stoppers are molded and assembled immediately under clean conditions and gamma sterilized. Filling is achieved by piercing the thermoplastic closure and then immediately resealing the puncture with a laser. COP and COC vials have been tested and used to replace glass in various pharmaceutical parenteral applications. This is because glass contains free alkali oxides and traces of metals and, at higher pH conditions, can undergo delamination, thus affecting the stability of the drug product (8,9). Proteins and peptides can be adsorbed on a glass surface and can either be denatured or become unavailable for treatment (10,11). Glass particles can promote protein particulate formation, and glass is also more likely to break under processing, storage or transportation of biopharmaceutical products, especially at lower temperatures. In these areas and more, plastic vials have made clear in-roads in the pharmaceutical drug delivery market.

Protein and peptide adsorption: Numerous studies have addressed the adsorption of proteins to packaging containers. This interaction of proteins and peptides with the surfaces of storage containers can result in their loss and destabilization (12 14). Although the amount bound is typically low, this problem can be acute at low protein concentration where a substantial portion of what is usually assumed to be solution-state protein may actually be adsorbed to the container walls. Although protein

Table 2 COP/COC Packaging Systems for Parenteral Delivery

Company	Trade name/type of cyclic olefin	Delivery system/sizes
Amcor/Alcan Packaging	COC	Vials 2 mL and 5 mL
Aseptic Technologies/Rexam	Crystal®/COC	Closed vials[a] 1 50 mL
Becton Dickinson	Sterifill®/Crystal Clear Polymer	PFS[b] 5 50 mL
Daikyo/West	Daikyo Crystal Zenith®/COP	PFS[b] 0.5 100 mL Vials[a] 0.5 1000 mL
Gerresheimer/Taisei Kako	Clearject®/COP	PFS[b] 1 20 mL
Rexam	MLx/COC, COP	Multilayer vials & bottles
Schott	Schott TopPac®/COC	PFS[b] 0.5 50 mL Vials 2 100 mL

[a] Presterilized vials and containers available

[b] Presterilized formats available

Abbreviations: COP, cyclic olefin polymer; COC, cyclic olefin copolymer; PFS, prefillable syringe.

binding is protein and formulation dependent, studies have shown a trend toward less protein adsorption to cyclic olefin containers. Burke et al. (15) compared glass vials with plastic vials made of polyester, PP and nylon for protein binding. Although no clear conclusion could be drawn on the binding characteristics of these primary packaging materials, it was observed that the degree of binding was highly protein-dependent. Qadry et al. (11) showed less protein binding to plastic CZ vials compared with type I glass, suggesting that the CZ vial is a potential candidate for an alternative material to the glass vial because of low affinity of proteins to bind to its surface. Eu et al. (16) compared the level of adsorption between glass and CZ vials and showed that a model protein preferentially adsorbed to glass vials compared with CZ vials. The authors used gold nano-particle staining techniques for a visual comparison of protein adsorbed to vial surfaces, but this technique does not permit quantitation of the amount of protein adsorbed to the surface. Waxman et al. (17) developed methods to quantitate protein adsorption on vial surfaces. One method uses the protein stain colloidal coomassie, which binds to protein adsorbed to vial surfaces and can be eluted and quantitated spectrophotometrically; the other method involves hydrolyzing the protein adsorbed *in situ* and quantitating the peptides released fluorometrically after reaction with fluorescamine. These approaches allow testing over a much broader range of protein concentrations without the use of radiolabeling. Using these methods, the authors confirmed that binding occurs rapidly and the amount of protein adsorbed per SVP vial is typically in microgram quantities. Protein adsorption to CZ vials was found to be independent of ionic strength, likely because of its hydrophobicity; in contrast adsorption to glass vials was inhibited with increasing ionic strength, indicating the effect of electrostatic interaction with glass containers. In our opinion, protein adsorption is clearly protein dependent, and testing needs to include glass and plastic containers with elastomer influence, before optimizing the drug formulation and container closure system.

Storage and transport at low temperatures: In the area of cell therapy, stem cell research holds significant promise for development of innovative therapies for many unmet or partially met disease treatments. As products enter clinical development stages, there is need for clean, clear, biocompatible, low extractables containers. The ideal vial-based system should be a suitable package to store and transport cell therapy products at lower temperatures; it should be suitable for commercial filling and meet pharmaceutical quality requirements. PP is a plastic resin that has been used for decades for various packaging applications including bottles, pouches, prefilled syringes, tubes and containers. Plastic resins have made minimal headway in the area of parenteral vials because of various quality attributes. A study investigated the use of CZ plastic vials for storing and shipping cell therapy products at low temperature (85°C) or cryopreserved (196°C) for six months using 0.5, 5.0 and 30 mL volume vials (18). Vials were tested for durability and integrity of a filled vial using a 1-m drop test, and for the ability to maintain viability and functionality of stem cells over the time of storage. No evidence of external damage was found on vial surfaces in the 1-m drop test. Post-thaw viability using dye exclusion assay was >95% and stored cells exhibited rapid recovery two hours post-thaw. Cultures were ~70% confluent within five to seven days, consistent with nonfrozen controls and indicative of functional recovery. CZ vials were durable and allowed for preservation and maintenance of cell viability and functionality, showing that these vials offer significant benefits to storing and transporting biological and biopharmaceutical products for storage, clinical and commercial applications.

Lyophilization and reconstitution: Cyclic olefin based plastics COC and CZ vials have been extensively studied for packaging lyophilized products. Freeze-drying in a plastic vial brings added advantages, especially when cytotoxic and biohazard products need to be packaged. Crystal technology, developed by Aseptic Technologies, applies the closed-vial technology for lyophilization (19) and for liquid fills. After filling closed vials using a piercing needle, a small disposable device called the penetrator reopens the orifice and, when the lyophilization chamber shelves move, the penetrator is pushed down, releasing the water vapor. Lyophilization of mannitol and arginine was studied in Daikyo's CZ vials and compared with molded glass and tubing glass vials. The crystallinity of mannitol in CZ vials was either greater or comparable to glass vials. There was thermal homogeneity within the CZ vial during the lyophilization cycle, providing more uniformity within the cake (20). Despite the fact that COC and CZ plastics provide a high moisture vapor barrier, it is always recommended that a secondary packaging barrier such as an aluminum pouch or a blister pouch with aluminum lidding and very low water vapor thermoformable film be used to assure adequate shelf-life protection for lyophilized products. For liquid fills in COC or COP, additional barriers are not necessary because of the low moisture vapor transmission rate of cyclic olefins.

Many drug candidates are marketed in lyophilized form to maintain shelf-life stability and require reconstitution prior to administration. Some of these products, including treatments for hemophilia, multiple sclerosis and autoimmune diseases, may be administered in a home environment. Traditional reconstitution requires the use of multiple vials and needles, which can prove to be complicated for patients or untrained personnel, and may increase chances for needle stick injuries. In recent years, there has been an increasing use of safer and more convenient reconstitution devices made out of plastics. These provide simple methods to reconstitute products without the use of needles and may also improve the effectiveness of the reconstitution process and compliance with the dosing regimen. There are several types of reconstitution systems designed to connect the drug container (typically a vial) to a diluent container (either a vial or a prefilled syringe). Plastic reconstitution devices are sterile, nonpyrogenic, biocompatible and fully supported by appropriate regulatory filings (21). They are designed for short-term contact with the drug product, and can be manufactured from a variety of medical grade plastic materials, such as PC and polyolefins, with the precise material selected on the basis of functional requirements. An example of a plastic reconstitution device, a vial adapter, is shown in Figure 4. For most vial adapters, and other components where a plastic spike is required, PC is used as it provides the appropriate balance of rigidity and sharpness to optimize spiking performance and attachment to the vial. Other materials, such as HDPE, can be used for components within the system where a stopcock system is required. These component devices are packaged in a rigid blister, often made from PET, to maintain sterility and to enable ease of handling and protection of the device during use. Plastic vial adapters can provide safe, easy to use and cost-effective diluent transfer to a lyophilized drug vial. The adapter snaps to the neck of the standard vial after the plastic button has been removed. A plastic spike pierces the stopper; needles are not

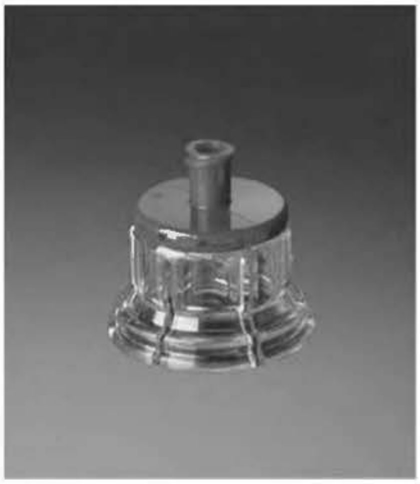


Figure 4 Vial adapter. *Source*: From Medimop Medical Projects Ltd.

used. Plastic vial-to-vial transfer systems also offer a similar level of simplicity and cost-effectiveness through a double-spike adapter that connects to the top of each vial (lyophilized drug and diluent). This is an ideal solution for connecting vials of different sizes. These advanced plastic reconstitution systems offer several benefits, including ease of use by patients and caregivers; protection against drug spray-back and accidental needle stick injuries; needleless reconstitution and transfer. They may also help drug manufacturers reduce the amount of overfill in the drug vial (22).

Process considerations: Glass vials are washed, depyrogenated and sterilized by heat before they are filled. Plastic containers cannot be heated to high temperatures for depyrogenation, therefore alternative methods are used. Plastic molding and packaging in environmentally controlled clean rooms usually produce products that have very low bioburden and low particulate level. Nonsterilized vials undergo water-for-injection rinses for depyrogenation, followed by sterilization using autoclave, radiation (gamma or e-beam) or ethylene oxide. All handling operations are designed to avoid scratching the vials' outer surfaces, as plastics have a tendency to scratch. To minimize scratching, care is usually taken not to stack vials too tightly in processing. During autoclave sterilization processing, hazing of the plastic walls is known to occur. This is where moisture gets trapped during processing and may take a few days to diffuse out, but the clarity and integrity of the vial is not compromised. Vial spacing during the autoclave sterilization process may help mitigate this effect. For vials in a ready-to-use format, vendors offer sterile vials and containers. Sterile vials or containers are nonpyrogenic and have a very low particulate level, and could be used to store and transport drug products as early as first-in-human studies. Most commercial filling companies can accommodate filling of COP and COC plastic vials if care is taken to accommodate the characteristics of plastic vials. During filling of plastic vials, guide rails and vial handling change parts should be covered with a material that will limit scratching of the vials. The speed of the filling line may also need to be adjusted to accommodate filling of the lighter plastic vials.

PREFILLABLE SYRINGE SYSTEMS

Market considerations: In the current global market, PFS comprise more than 2.0 billion syringes per year in development and use. The origin of the prefilled syringes' rise as the preferred container was an extremely successful market introduction of syringes for heparins by Sanofi and Rhone Poulene-Rorer (Sanofi-Aventis) in Europe in the early 1980s. The PFS market has now exploded because of several factors: the growth of biopharmaceuticals; the need to eliminate overfills; precision of delivery volume; convenience of delivery, cost-effectiveness; elimination of dosage errors or a combination of these factors (23 26). Glass continues to dominate the PFS markets with a significant market share; however, plastic PFS are beginning to make advances, especially where glass has been unsuitable as a delivery system. PFS have been in use as larger volume containers for x-ray contrast media or medical devices such as hyaluronic acid derivatives (23). In the last decade, however, pharmaceutical drug products have been approved for use with prefillable plastic syringes, including a new chemical entity for oncology and a peptide drug product for the treatment of osteoporosis (Table 3).

Table 3 Global Regulatory Approvals of Drug Products in Cyclic Olefin Polymers/Cyclic Olefin Copolymers

Therapeutic area	Plastic packaging	Approvals
Anemia	Cyclic Olefin	Japan
Osteoporosis/Oncology	CZ vials	United States, Europe, Japan
Antifungal	CZ vial	Japan
Osteoporosis	CZ syringe	Japan
Radiology	CZ syringe	Japan
WFI product (for thrombolytic drug)	TopPac® syringes	Europe

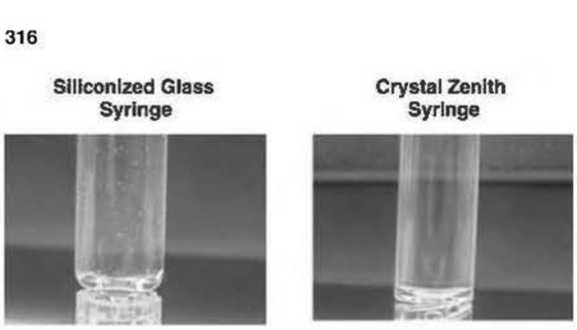
Abbreviation: CZ, Daikyo Crystal Zenith®.

Although not reaching the adoption level of glass syringes, plastics syringe systems continue to gain strong acceptance from pharmaceutical manufacturers because of recent improvements in design, composition and manufacture. Plastic syringes were historically made out of PP, however, recent developments in the area of thermoelastic polymers, such as cyclic olefins, have made substantial headway in the use of plastics as a PFS system. COP is as clear as glass, has low extractables, is less reactive and has better barrier properties compared with PP. Multiple vendors offer different sizes of syringes in sterile nested configuration or as nonsterile bulk syringes. Cyclic olefin plastic barrels are formed by injection molding under clean conditions and assembled in similar conditions, primarily to maintain a high level of cleanliness. Plastic syringes are sterilized either by autoclave, radiation (gamma or electron beam) or by ethylene oxide, but not by dry heat, and are offered as assembled sterile syringes that are ready for filling. The molding process also provides a greater degree of flexibility to include design features such as a plastic finger grip that can be combined with a back stop to prevent the piston being pulled out of the barrel.

To meet the need for lubricity and sealability, syringe manufacturers use silicone to coat the glass barrels and elastomer components. Silicone facilitates ease of movement of pistons in filling and stoppering equipment, and allows pistons to glide smoothly on activation of syringes. Silicone, however, can interact with drug formulation components (27,28). Recent developments to minimize free silicone include baking silicone at high heat onto the glass barrels, thereby minimizing the amount of free silicone that can interact with drug product. Advances in elastomer closure technologies have produced closures that do not require siliconization because of a special polymer lamination applied to the outer surface of the piston, thereby offering a silicone oil free PFS system such as the Daikyo CZ syringe system. The syringe system includes a plastic COP barrel, nozzle cap and piston laminated with a fluoropolymer lamination, Flurotec®, and requires no silicone for consistent functionality. Flurotec is a lamination technology using copolymer film of polyethylene tetrafluoroethylene (PTFE) or ethylene tetrafluoroethylene (ETFE). Helvoet (Omniflex® 3G) pistons also have a fluoro-polymer coating, however, these typically are coated with a sprayed-on polyvinylidene fluoride (PVDF) and will need siliconization for use with glass or plastic barrels. Use of these coated stoppers provides lubricity for machinability and reduces piston clumping in feeder bowls. Additional benefits, depending on the coating used, include a decrease in particle generation and a reduction of extractables from the elastomer (27,29).

Improving protein stability: Growth in the pharmaceutical industry is expected to be driven by biotechnology products and vaccines. This will be associated with significant challenges in the formulation development of proteins such as monoclonal antibodies, as they are typically administered in high doses. High-concentration proteins have a propensity to interact with each other and with the packaging components and cause protein instability, especially when the volume of delivery is approximately 1 mL. Challenges with glass PFS typically encompass breakage, presence of particulates, glass reactivity to the drug product and potential leachables including silicone, tungsten and adhesive. A plastic PFS offers options to solve such challenges. A plastic PFS can eliminate silicone, tungsten and adhesive, depending on the quality attributes of the entire prefillable system. For instance, the CZ insert needle system uses no silicone for syringe functionality, no tungsten (commonly used during the glass syringe forming process) and no adhesive (commonly used to hold the staked needle in place).

There are reports that the detachment of silicone oil in water-filled syringes is possible (30) and can result in particulate matter and clouding phenomenon. Silicone oil interaction has been suspected as being responsible for aggregation in protein pharmaceuticals. Several publications in the 1980s have discussed this issue, especially with regard to the aggregation of insulin in disposable siliconized plastic syringes (31 33). Surfactants such as polysorbates have been used extensively to prevent/inhibit protein surface adsorption and aggregation under various processing conditions (34,35). One consequence with using polysorbates in protein preparations


Siliconized Glass Syringe

Crystal Zenith Syringe

Figure 5 Aggregates in siliconized syringes and silicone free syringes.

is their potentially adverse effect on protein stability, including the oxidative damage of the residual peroxides in Tweens, which are generated during processing or storage (36). This can pose a serious problem affecting the shelf-life of products. Polysorbates and their concentration should be selected carefully. In addition, the choice of a suitable container will help mitigate significant risks of protein aggregation caused by silicone oil. The propensity of proteins to aggregate when silicone oil is present in formulation was further investigated by Esfandairy et al. (37). Silicone oil induced aggregation of proteins was studied on silicone oil free plastic syringe systems and siliconized glass PFS systems. The study included model proteins at low concentrations of 0.35 mg/mL to as high as 25 mg/mL. Although no unambiguous generalization was drawn at lower concentration, there was a clear effect at protein concentrations as high as 25 mg/mL. Effects on protein aggregation with silicone oil were observed during air shipment of samples, caused by effects of agitation and vibration. The study showed that the extent of aggregation in silicone oil free CZ syringes was less compared with siliconized glass syringes under the conditions examined (Fig. 5). The study recommended that the susceptibility of therapeutic proteins to silicone oil induced aggregation be investigated on a suitable container closure system before finalizing stabilized formulations and container selection.

Various methods are used to siliconize syringes, including stationary nozzles and diving nozzles. Recent studies have shown that (16) silicone oil distribution is often nonuniform, leaving certain areas of the syringe surface without any silicone oil. The low or inconsistent silicone oil coating can have a significant impact on the piston travel/glide forces, especially in the use of autoinjectors. In 2006, lots of Neulasta® delivered by an autoinjector containing a glass PFS were recalled in a number of European countries because of problems with slow or incomplete delivery of the drug (38). Areas of nonuniformity cause travel forces to increase, causing failure or incomplete injection. In addition, there has been significant attention to tungsten as a leachable present in glass PFS. These reports discuss tungsten-based particulate matter leaching and interacting with the protein drug product (39). Tungsten pins are typically used to keep the fluid path open at the nozzle end of the syringe at around 1200°C during the glass syringe forming process. Upon cooling, a needle is staked-in with adhesive, to make a glass PFS with a staked needle. The residual tungsten had migrated into the drug product and caused the protein to form protein-tungsten aggregates. Although this appears to be protein specific, it is important to test for protein-tungsten interaction at an early stage of drug development. In another case, a residue was observed in a PFS during the manufacturer's inspection. Upon investigation, the material was identified as poly (metaxylylene adipamide), a component of the glass fiber pin use by the syringe developer during the needle assembly and curing process (40). Such concerns may be mitigated with the use of COP/COC syringes. Silicone oil free CZ syringes have been shown to have consistent travel forces over time and temperature. The dimensional tolerance of plastic syringes and consistency of syringe functionality will provide a predictable operation of a drug product filled autoinjector. CZ syringe systems have no tungsten as a leachable because the needle is insert-molded, avoiding the need for tungsten pins and adhesive, which are typically used with glass staked-needle syringes. The manufacturer has developed a PFS system intended for biopharmaceutical drug delivery that is free of silicone oil, tungsten and adhesive (41).

Process considerations: In the current market environment, presterilized, ready-to-fill syringes are increasingly more prevalent. PFS are now available in sterile and ready-to-used formats. As glass PFS are already being filled using tubs, a switch to PFS in a similar tub and nest configuration has been achieved using the same filling machines, with minor modification and change parts to accommodate plastics. Most commercial filling companies can accommodate plastic syringes. The control of dimensional tolerances of plastic syringes far exceeds that of glass syringes and, because they are less prone to breakage and shattering, plastic prefilled syringes are generally easy substitutions for glass PFS on modern filling/processing equipment. There are, however, some physical differences between glass and plastic that should be considered before running plastic PFS on a filling/processing line designed for glass PFS. Plastic syringes are prone to scratches and cosmetic defects from contact with metal surfaces in processing equipment and the weight of plastic PFS is less than their glass equivalents. Scratching may create an unacceptable level of cosmetic defects. Lighter weight syringes can cause problems when gravity is responsible for syringes settling into place in processing equipment. The issues of weight and scratching often manifest themselves when metal centering devices are used to hold and center PFS during filling and stoppering processes. These problems can be overcome by reengineering some parts of filling and processing equipment or by running equipment at slower speeds. It is expected that, as the use of plastic PFS becomes more prevalent, manufacturers of filling/processing equipment will design equipment that performs equally well with both glass and plastic PFS.

There are various processes for filling and stoppering PFS. These include filling and stoppering using vent-tube, or vacuum fill or/and stopper placement. Vent-tube is more commonly used for uncoated or partially coated pistons intended for glass and plastic PFS. For coated pistons, vacuum placement works well as the procedure uses differential pressure rather than force to eliminate wrinkling of the lamination. Vacuum placement is particularly important for laminated pistons, especially in CZ syringe systems, which use a piston that is coated on the drug product contact and syringe barrel contact surfaces. The piston provides lubricity for efficient piston release and consistency of travel forces for a silicone oil free system. An option offered at Hyaluron Contract Manufacturing, Burlington, Massachusetts, for filling PFS, BUBBLE-FREE FILLING®, uses online vacuum filling and online vacuum stoppering (42). The primary advantage is the reduction of the air bubbles that exist between the product and the stopper in traditionally filled syringes. This may help mitigate concerns regarding oxidation of the product.

LARGE-VOLUME PARENTERALS

LVP refer to sterile diluents, electrolytes, irrigating fluids, blood derivatives, nutritional preparations and premixed injectable drugs administered in quantities of over 100 mL. LVP are packaged in semi-rigid plastic containers, flexible minibags and, to a lesser extent, glass containers. Three major global manufacturers of LVP include Baxter, B. Braun and Hospira. The sterile formulation of LVP necessitates the use of containers with good barrier properties and sizes of semi-rigid plastic IV containers range from 250 mL for biologicals and nutritionals up to 4 L for standard diluents (such as sodium chloride and dextrose).

Minibags are used for administering lower-volume parenteral admixtures, and most premixed IV solutions are packaged in specially designed minibags. IV minibags usually contain 50- or 100-mL volumes of solution and are made of PETG, PP and various polyethylene-based coextrusion. These containers provide a sterile format consisting of a drug mixed with an appropriate diluent solution. Premixed minibags eliminate the need for independent admixture preparation and provide significant time, labor saving and waste reduction advantages. Most major parenteral drugs are now available in this format, including drugs for antibiotic, analgesic, anticonvulsant, cardiovascular, psychotherapeutic and respiratory preparations. Some solutions packaged in the container must be stored frozen and thawed no more than 24 hours prior to use.

Historically, PVC was the leading material employed in the production of LVP configurations. However, this trend has changed because of potentially adverse patient reactions to a plasticizer used to stabilize the resin. Known as DEHP, the plasticizer has been linked to infertility and hormonal imbalances in laboratory animals. Regulatory authorities have recommended that all medical products based on PVC and DEHP be either adapted to alternative materials or include a label warning about the plasticizer. In response, the producers of IV solutions have adopted newer plastics for their containers. B. Braun Medical eliminated the used of PVC in IV packaging. The company's Excel® and PAB® IV containers now include specialized PP materials. Newer, higher-grade plastics, such as PETG copolyester, are being used for minibag applications to keep solutions stable, including Baxter's and Hospira's products. Baxter International recently introduced Buminate® human albumin solution in a Galaxy® minibag that is composed of proprietary, high-barrier plastic film. The new Galaxy package can provide a shelf-life of two years and eliminates the need for preparing admixtures in hospital pharmacies. Hospira's ADD-Vantage® system is a specially designed diluent container that connects to a vial. Once the vial is affixed to the container, the active drug blends with the diluent and creates the finished IV solution. The ADD-Vantage system allows the IV solution to be mixed directly at the site of administration. Another innovative IV minibag system is the Duplex® Drug Delivery System developed by B. Braun. Duplex is a dual-compartment flexible plastic IV bag that stores unit dosages of drug powder and diluent separately in the same container. The health care professional squeezes the bag to break the quick-release seal, mixing the drug and diluent just prior to administration. Designed to simplify the intravenous delivery of antibiotics, the Duplex container reduces product waste, eliminates the use of vials from the preparatory process, and is equipped with a standard linear bar code to reduce dosage errors and track inventory.

X-ray contrast media is also packaged in a range of volumes from 50 to 500 mL in both plastic and glass containers, with the 500 mL containers labeled as pharmacy bulk packages. PP prefilled syringes and prefilled PP cartridges designed to fit a specific range of power injectors for computed tomography are available (43). Another design of a prefilled cartridge called REDIFLOW™ is available in a clear plastic to fit a second range of power injectors for computed tomography as well as PP bottles (44).

PLASTICS AS DISPOSABLE SYSTEMS FOR BIOPROCESSING

Market considerations: Plastic packaging systems for LVP drugs are facing increasing scrutiny. All packaging systems, stainless steel or plastic, need to provide and meet the same requirement for protection, compatibility and safety as those used for SVP (45). This section addresses the use of plastics as disposable bags in packaging large-volume drug substances or drug products in bioprocess development and fill/finish operations (46,47).

According to the report released by the Tufts Center for the Study of Drug Development, Outlook 2009 (48), there are more than 200 new monoclonal antibodies in development worldwide, and the FDA has approved 22 monoclonal antibodies. To support development of these biologics, the biopharmaceutical manufacturing industry is rapidly adapting to disposable systems. Single-use bioprocess systems referred to as disposables have become common in the industry. Disposable systems have gained increased acceptance for manufacturing-scale storage and processing of recombinant proteins and monoclonal antibodies in liquid and frozen forms (49,50). This is driven primarily by the key benefits plastic disposable containers offer over stainless steel containers. These include reduced capital expenses (stainless steel vessels, cleaning and sterilization validations), minimizing cross contamination, flexibility in manufacturing and easier scale up (51,52).

Disposable technology employs a multitude of plastics to customize processing and may include bags, filters and tubing. Plastic materials that make up the critical

Table 4 Commonly Used Plastics in Disposable Systems

Disposable bags	Polyethylene, ethylvinyl acetate, PVDF
Filters	PTFE, polypropylene, PVDF
Tubing	Silicone, PTFE, PVDF

Abbreviations: PVDF, polyvinylidene fluoride; PTFE, polyethylene tetrafluoroethylene.

components of a disposable system include filters (e.g., Millipore, Sartorius, Pall, GE Healthcare), tubing (e.g., Amesil, Saint Gobain), and disposable bags (e.g., Hyclone, Stedim, TCTech, Pall). Disposable bags are larger volume containers that are used for large volumes of drug substances or products and have the greatest dwell time of product exposure. These bags are used in upstream and downstream bioprocessing and in fill/finish operations, examples include media preparation, bioreactor, storage and transportation. Multilayer bags are typically used and are intended to maintain product integrity. These bags provide gas and moisture barrier properties, functionality after sterilization, durability and biocompatibility (Table 4). Very few materials possess a balance of properties in one layer and PVDF film may be the best solution (47). The outer layer of a multilayer bag provides durability, and many materials are used with varying thickness. These materials are made up of nylon, polyesters, ethylvinyl acetate (EVA) and polyethylenes. As a sandwich layer, ethyvinyl alcohol (EVOH) is commonly used. EVOH has extremely low gas permeation and excellent barrier characteristics. Because it has a propensity to absorb moisture and lose its barrier property, it is sandwiched in a multilayer bag. LDPE is commonly used as the drug contact layer because of its good chemical compatibility profile. While EVA films are typically considered superior as the product contact layer, there are limitations to large-scale manufacturing of EVA film, and, consequently, LDPE becomes a good alternative, especially with three-dimensional bags such as those used in disposable mixing applications.

Many factors are usually considered during the design phase when choosing a disposable bag. Two important questions to be addressed are: Is the plastic polymer safe and is it compatible with the solution it is in contact with? Several facets related to the qualification and selection of a disposable container must be considered to address these questions. This includes a validation package from the vendor with information related to the materials of construction, sterility, USP plastic class VI data, extractables, heavy metals, particulates, pyrogens and cytotoxicity testing from the vendor. This information in combination with knowledge of the drug substance or drug product processing that may include processing volumes, chemical stability, compatibility, number of campaigns, formulation components, processing conditions such as temperature, pressure and, most importantly, extractable and leachable considerations can provide insights into the choice of disposable bag for bioprocessing. The primary considerations should include:

Chemical resistance study: Chemical compatibility studies should be conducted to evaluate the choice of a single-use container prior to its selection. The tests can include weight loss, clarity, visual inspection, drop test, tensile strength, thickness of the film and testing using various solvent systems including buffers, organic solvents or other components that may be intended for drug product development. For most aqueous formulations, the plastics (e.g., LDPE, HDPE, PP, etc.) have an acceptable compatibility profile. However, organic solvent usage may cause incompatibility issues. Emerging disposable systems bags such as PVDF, which has a chemical compatibility profile similar to Teflon®, may offer options for accommodating formulations based on organic solvents.

Protein adsorption: Single-use systems are increasingly prevalent in downstream processing, final formulation development and in fill/finish of protein solutions. These systems gained acceptance for storage and processing at manufacturing scale of recombinant proteins and monoclonal antibodies in liquid or frozen forms. The container-protein interactions may include protein adsorption onto the plastic container surfaces. The major driving forces influencing adsorption of protein are hydrophobic and electrostatic interactions. These interactions are responsible for nonspecific protein binding on a variety of surfaces. Interaction factors between plastic surface and protein could be affected by the physical nature of the surface (material surface or any coating), product formulation (pH, ionic strength, surfactant, etc.), storage conditions (temperature and contact time) and the concentration and conformational properties of the protein. Studies have shown a low binding level of model proteins on plastic polymeric surfaces compared with borosilicate glass surfaces. It is important to evaluate plastics using specific protein binding assays under various processing conditions, using large surface-to-volume ratios to determine their acceptability (53).

Extractables and leachables: The release of compounds from the plastic may affect product quality such as plasticizers, stabilizers or solvents. Regulations mandate that the equipment and materials used in the manufacture of pharmaceuticals should not alter the safety, efficacy and potency of the final drug product. An evaluation of potential extractables is required for plastic disposable bags to ensure compliance. Extractables are substances that can be extracted from a plastic using solvent or extraction conditions that are expected to be more aggressive than the processing conditions intended. Leachables are substances that could be present in the finished product because of interactions between plastics and the drug product during the products shelf-life. The suppliers of the plastic bags or components should provide a full and complete potential extractables list which could be used to evaluate product suitability with the plastic disposable bag (54).

Sterile barrier integrity: Maintaining integrity of a disposable device is critical to protect the product from microbial contamination. When plastic bags or components are provided as sterile, the integrity of these products must be demonstrated. Container closure validation can be performed to reduce the risk of compromise. These tests may include helium leak testing, pressure testing, dye ingress or microbial ingress challenges. Guidance documents from the FDA and European Medicines Agency (EMEA) can help to define the level of validation and qualification necessary for the safety of the single-use systems. These include the FDA's guidance document issued in May 1999, "Container-Closure Systems for Packaging Human Drugs and Biologics" (45) and EMEA's guidelines on plastic primary packaging materials (55).

QUALITY AND REGULATORY CONSIDERATIONS

There are numerous plastic containers that have been used for parenteral applications, including drug products in cyclic olefin containers that have been approved for marketing in the United States, Europe and Japan (Table 3). Guidance documents from FDA and EMEA help define requirements and the level of validation and qualification needed. This guidance has been universal to encompass all plastic containers for SVP or LVP, including vials, PFS or flexible bags. The FDA document "Container Closure Systems for Packaging Human Drugs and Biologics" provides the fundamental guidance on container closure systems, including plastic materials (45). The United States has a drug master file system (DMF) in which companies provide confidential information on the manufacturing and the composition of the plastic in a type III packaging material DMF and is incorporated into a letter of authorization for referencing the DMF upon FDA review. Canada has a similar DMF system, except that packaging materials are listed in a type II DMF. In Europe, the EMEA limits the information contained in a DMF to drug substances; therefore, the drug manufacturer will usually provide the required information on the packaging system. Guidelines for plastic containers can be found in the newly revised EMEA's Guideline on Plastic Primary Packaging (55). Both Ph.Eur. and USP have chapters referencing plastic materials and plastic packaging. Ph.Eur. section 3.1 has detailed chapters on various plastics including "polyolefins," and Ph.Eur. 3.2 specifically focuses on plastic containers (56,57). USP combines guidelines for plastic containers and plastic materials in chapter <661> (58). With respect to biocompatibility, both in vitro and in vivo biological reactivity needs to be performed on plastic containers (59,60). The quality-conscious Japanese market has seen the plastic market grow significantly for SVP. Mitigation or elimination of particulates or defects, safety, break resistance and clarity are clearly the drivers for using plastics in Japan. Key JP guidance is described under General Tests Processes and Apparatus, 7.02 Test Methods for Plastic Containers and General Information 17, Plastic Containers for Pharmaceutical Products (61).

SUMMARY

Application of plastics for parenteral delivery is expected to grow in years to come. Although PP material is more commonly used because of its availability and cost-effectiveness, there has been a recent surge in the use of superior plastics, the cyclic olefins, for parenteral delivery. The features of cyclic olefins are seen very favorable when packaging SVPs, highlighted by properties such as break resistance, glass-like transparency, better barrier properties compared with other plastics and its biocompatibility. However these features need to be balanced with the needs of a drug product, especially in the areas of oxygen or moisture sensitivities, where secondary packaging may help reduce such risks. Plastics are also favored because of their moldability and tight dimensional tolerance and can lead to newer design integrations. Examples include front finger grips, larger flanges and back stops for syringes. This capability is especially important because the home health care market is a growing segment. Many drug products are produced with the intention of being used in a home setting. Material flexibility also allows the same resin to be used in an assortment of designs, from vials through PFS systems, without substantial chemistry differences. Recently cyclic olefin syringes have become available in sterile assembled formats for ease of filling, similar to that of glass syringe packaging, making it easier for drug manufacturers to switch to plastics. Similarly sterile and nonsterile plastic vials and containers are also available.

Plastic container systems can also play a significant role in influencing the stability of a drug product. For example, they are used with drug products that would otherwise delaminate glass or with water-for-injection products to maintain pH. Recent advances in plastic PFS systems include developments in silicone-oil free and tungsten-free syringe systems that can help mitigate or eliminate any potential interaction of leachables from a packaging system. Formulators and package engineers now have more options to evaluate and optimize drug formulation with suitable packaging components at early stages of drug development. Protecting the drug product in a package that does not break or crack is a substantial benefit, especially with biological products that need low temperature storage and transport. Plastic vials are now considered in these areas. In addition, availability of plastic cartridges and plastic dual chambered syringe systems for liquid-liquid or lyophilized powder-liquid systems clearly illustrates the ability of vendors to offer such designs for various drug delivery applications. For large-volume packaging and processing of bulk drug products, plastic disposable bags are being considered. Clearly plastic disposable bags offer many benefits over stainless steel containers in downstream bioprocessing, including fill/finish operations; however, due diligence is a must for the right choice of plastic for the product. Plastics will increasingly be utilized throughout the entire total supply chain of pharmaceuticals and provide opportunities for total life cycle containment of pharmaceutical products. These opportunities can allow for the lowest total cost of ownership to be provided with plastic packaging materials.

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13 Elastomeric closures for parenterals

Renaud Janssen

SUMMARY

The present chapter in this review work intends to give insight into elastomeric closures that are used for parenterals. The single most important reason why elastomeric materials are used for closures for parenterals is that the elasticity of such materials allows for preservation of the sterility of the packaged drug, by ensuring a tight seal between the closure and the container, and by ensuring adequate resealing of the closure after penetration with a needle or with a spike in cases where this is applicable.

Of course sealing and resealing are not the only features that characterize elastomeric materials. Elastomeric closures also have benefits in that they are able to give a property profile that is an ideal combination of physical, chemical, functional and biological performance, combined with microbiological and particulate cleanliness.

This chapter is an endeavor to give the reader insight into this complex system of properties and requirements.

THE MANUFACTURING PROCESS FOR ELASTOMERIC CLOSURES

The text below describes the operation of a typical modern elastomeric closure manufacturing plant. In any such plant, irrespective of the name of the company, the major steps in pharmaceutical rubber stopper manufacturing will consist of weighing according to a recipe, mixing, preforming, molding, die-trimming, washing, drying and packing (Fig. 1).

Raw Materials

The basis for the manufacturing of rubber closures is a so-called rubber compound. It is composed of a number of raw materials.

Raw materials are quarantined upon receipt and there is a system in place for testing of raw materials for identity and purity according to specific procedures and specifications.

Upon acceptance by the control laboratory, raw materials are released for production and a raw material lot number is assigned. All relevant data are stored in a computerized raw material lot file. There are provisions in the manufacturer's quality system to protect against inadvertent use of nonreleased raw material lots.

Mixing and Preforming

Individual rubber compound batches are composed by combining the required amounts of each rubber ingredient in accordance with a formulation sheet ("recipe"). The ingredient's weight accuracy and lot numbers are stored in the compound batch file. Each weighed quantity is duly identified.

Weighing of the ingredients and composition of the individual compound batches take place in specially equipped rooms, designed for cleanliness and logical material flow. Large-volume ingredients such as fillers may be stored in silos in which case they are automatically weighed and delivered directly to the mixer, thus largely reducing the potential for dust and contributing to cleanliness of the manufacturing environment.

The compound ingredients are mixed in a Banbury type mixer. A Banbury type of mixer consists of an extremely robust mechanical chamber in which the rubber ingredients are mixed by the action of cooled cylindrical rolls that rotate into each other. Prior to introducing the ingredients into the mixer, their identity is verified.

The mixing process is highly automated and entirely computer controlled, as it functions according to a predetermined "mixing recipe." The mixer parameters that are important for the quality and the properties of the mixed material typically are constantly monitored and recorded.

At the end of the mixing cycle, the rubber compound batch is transferred onto an open mill where it is cooled and further homogenized. Next the rubber compound batches are

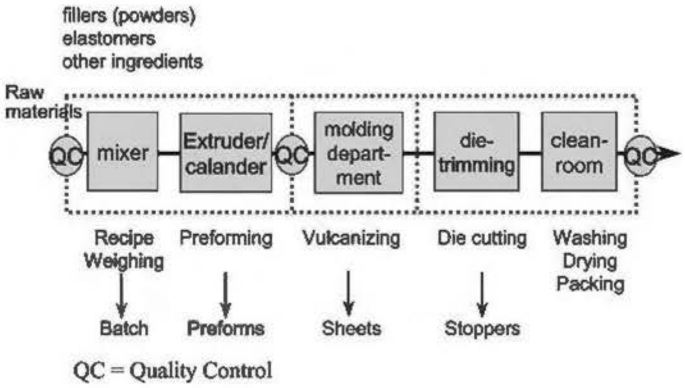

Figure 1 Elastomeric closure manufacturing process.

shaped into "preforms" with the size and weight required for molding in a particular mold. The preforming operation may have different forms. It may consist of passing the mixed and milled rubber through an extruder and cutting the extrudate into bricks of a well-defined form and weight. Alternatively it may consist of a calandering operation where the rubber coming from the calendar is cut into slabs that again have a well-defined shape and weight. At the stage of mixing or preforming typically a sample of each compound batch is checked for correct vulcanization properties by means of a rheometer test. Furthermore, a sample is sent to the laboratory for testing of physical and chemical properties. All data are stored in the compound batch file and are fully traceable.

Molding

Both injection and compression technologies may be used for molding rubber closures. The choice depends on the technical requirements and characteristics of the products.

The rubber preforms are heated under high pressure in multicavity molds. During this process the rubber vulcanizes. In the vulcanization process, by the use of cross-linking agents that are contained in the rubber compound, chemical bonds are formed between individual polymer molecules that form the elastomeric base of the rubber. It is only at the stage of molding that the rubber turns from a plastic into an elastic material, and that it acquires its required shape in the form of a vial stopper, of a plunger for a cartridge or a prefilled syringe, or of any other geometrical form that is intended to shape the rubber in.

The products leave the molds in the form of "sheets," each carrying many closures. The operators performing the molding operation typically examine the quality of the molded sheets at this stage, which marks the first quality check of the elastomeric components.

The use of modern, proprietary compression and injection molding technology, combined with proprietary mold construction technology, results in rubber closures with narrow tolerances and stable nominal dimensions.

Die-Trimming

The sheets with the products are then die-trimmed to result in individualized stoppers. This operation may take place in the immediate vicinity of the molding press or in a separate area that is designed for higher cleanliness. Die-trimming of elastomeric closures requires a trimming agent, which is typically a silicone emulsion, that is then removed by rinsing the freshly die-trimmed stoppers or, in case this is not present, in the next manufacturing step, which is washing.

Washing Process for Elastomeric Closures

The die-trimmed closures are transferred to the washing and posttreatment area. At present time rubber closures for parenteral applications are always washed, regardless of the closure manufacturer.

Washing of rubber closures typically is combined with siliconization. Siliconization of rubber closures is necessary to overcome the stickiness that is inherent to typical rubber formulations that are used for parenteral stoppers. Washing is performed to improve the state of microbiological and particulate cleanliness of the stoppers. Washing and siliconization may take place in washing equipment of various types. Very often, rotating drum type equipment is used for washing, siliconization and drying. However, the state of the art practice is that closures are washed in a pass-through machine. Loading of the closures takes place at the "dirty" side of the machine, while unloading is foreseen at the "clean" side in a room with a controlled state of cleanliness.

Various procedures exist for washing of parenteral closures. Every stopper manufacturer has its own process. More on stopper washing and siliconization can be found in a later paragraph of this chapter. At any rate washing is followed by drying with air of controlled cleanliness.

Packaging

After drying, the rubber closures are immediately packed in clean polyethylene (PE) bags, and sent out of the washing area into the packaging area where the bags are put into cardboard or plastic boxes. The plastic bags and the boxes are labeled with identification data such as product and compound code, lot number, packaging date and information on the final treatment.

In case the closures are manufactured "ready for sterilization" or "ready to use," packing takes place in dedicated functional ready-for-sterilization (RfS) or ready-to-use (RtU) bags. RfS and RtU bags are overwrapped with protective plastic bags before putting them into the cardboard or plastic boxes.

Classification of Manufacturing Environment and Environmental Controls

The manufacturing of rubber still to a large extent is an industrial process, especially in the first steps of mixing and to a lesser extent in molding. Throughout the manufacturing process it is usual that the closure manufacturer progressively implements measures to work in cleaner areas and to protect the products or intermediates from contact with the environment, including the manufacturing personnel.

In practice this comes down to implementing systematic cleaning programs in all areas, sound gowning procedures for operators, for their supervisory personnel and for plant visitors, and appropriate measures to protect products from environmental contamination. In the initial manufacturing steps of mixing, molding, and die-trimming it is not common that a closure manufacturer will classify the manufacturing areas. Exceptions to this are for new plants that are built from scratch. For washing and packaging areas, though, it is common that these areas are classified.

Classification may be done in various ways. Whereas in the past it was most common to speak of class 100 or class 1000 or class 10,000 or . . . in terms of the U.S. Federal Standard 209, today classification is mostly done in terms of International Standardization Organisation (ISO) 14644-1, "Cleanrooms and associated controlled environments Part 1: Classification of air cleanliness" and/or in terms of the FDA Guidance for Industry, "Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practice" or the EU Guidelines to Good Manufacturing Practice, Annex 1, "Manufacture of Sterile Medicinal Products" Grade A/B/C/D classification. It may be noteworthy to verify whether a manufacturer claims a classification for his manufacturing areas "at rest" or "in operation."

Classification of manufacturing areas needs to go hand in hand with the implementation of a monitoring system to demonstrate not only initial compliance but also continuous compliance. This system to demonstrate continuous compliance is then based on a sound rationale for measuring nonviable and viable airborne particulates, complemented by measurements of surface microbiological cleanliness, and in the highest degree of sophistication also of contamination of personnel gowning. Since in the final washing of closures for parenteral application, modern standards require that water of defined purity such as purified water and water for injection is used, also monitoring of the compliance of the various water types will need to be part of the manufacturer's total monitoring system.

PARENTERAL CLOSURE TYPES AND DESIGNS

The present part of this chapter gives an overview of the most important and common types and designs of closures that are used as *primary* packaging in parenteral applications. No attempt is made to review components that are used as secondary packaging such as aluminum or aluminum/plastic crimp caps. Since some closure *designs* may be proprietary to the closure manufacturer or end-user, it is impossible to put together an exhaustive listing here. This will not preclude though that the overview below is as complete as possible pertaining to closure *types*.

Stoppers for Vials and Bottles

Closures for Serum Vials

These closures are the rubber stoppers that are used for closing glass or plastic vials or bottles stemming from liquid or dry powder fills (Fig. 2).

These closures consist of a flange having a larger diameter and a plug part having a smaller diameter. The plug part fits into the vial neck while the flange part rests on the rim of the vial.

Closures in this category are usually subdivided by their size. These subdivisions include 13-mm stoppers and 20-mm stoppers for small-volume parenterals (SVP), and 28-, 29-, and 32-mm stoppers for large-volume parenterals (LVP). These sizes do not correspond with any diameter of the closure itself, however they indicate the largest diameter of the vial neck. For example, a 20-mm stopper is used for closing a vial with 20 mm as the outer diameter of the vial neck, while the flange diameter of the stopper typically is between 18.8 and 19.1 mm.

Stoppers in this category have two further features.

1. On the top of the flange there is an antistick marking. Rubber always has a tendency to stick, especially the type of rubbers that most parenteral stoppers are made of. The purpose of the antistick marking is to prevent the two large flat flange surfaces of two different stoppers from sticking together during storage of the stoppers, during steam sterilization and during filling operations at the pharmaceutical company. A well-studied design of antistick markings greatly helps in preventing clumping of stoppers in all these stages.

   The antistick markings also often delineate the target area of the stopper, that is, the area that is intended to be pierced with a needle or a spike.
2. The presence or absence of a constriction just underneath the flange. This constriction is called "blowback." Its role is to fit into a corresponding protruding part of the inner rim of the vial mouth so as to prevent the stopper after placement from rising and popping out of the vial neck. In this respect one also speaks of a "no-pop" feature or a no-pop stopper.

Significant dimensions of this type of stopper to consider are as follows:

1. Flange diameter: obviously this diameter has to be compatible with the outer diameter of the vial neck.
2. Plug diameter: obviously it has to adequately match the inner diameter of the vial neck and in forthcoming case its blowback.

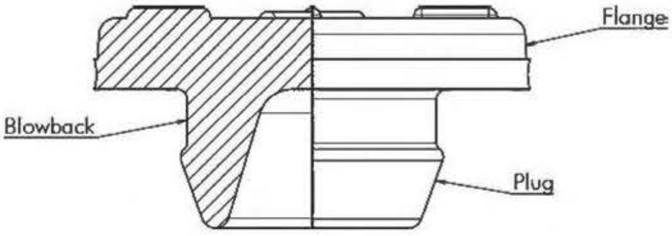

Figure 2 Section of a serum stopper with blowback.

3. Flange thickness: this dimension may be of primary importance for machineability of the stoppers on filling lines. Flange thickness should be well controlled by the stopper manufacturer.
4. Total stopper height: depending on the filling line this dimension can play an important role in stopper machineability.
5. Penetration thickness (the thickness of the stopper in the penetration area): this thickness is one of the contributing factors in determining the coring, the resealing and the penetration behavior of the stopper. Additionally, this thickness after capping of the vial determines the permeability to gases of the stopper/vial/cap combination. Given a certain rubber material, higher penetration thicknesses can lead to higher resistance to permeation of air and moisture into the vial and thus into the drug.

All these dimensions are expressed as nominal values and respective tolerances. Both are partly normalized in ISO standards such as ISO 8362-2 (closures for injection vials) and ISO 8536-2 (closures for infusion bottles).

For design purposes it is necessary to understand that tolerances of rubber parts cannot be as tight as for plastic parts. Dimensions on rubber parts as per ISO 3302-1 can be subdivided into dimensions that are determined by the rubber mold and dimensions that are determined by the rubber molding process. The former ones are tighter than the process related dimensional tolerances, however they are still larger in comparison with what is usual for plastics. With respect to serum stoppers, diameters are mold related, while dimensions such as flange thickness, penetration thickness and total height are process related.

A frequently asked question is where the effective seal between stopper and vial takes place, or which matching surfaces of stopper and vial are responsible for container/closure integrity.

For capped vials, or where under the influence of a crimp cap the underside of the stopper flange exerts a force on the top of the rim of the vial neck, it is this interface (underside flange / top of vial neck) that constitutes the seal. The permanent seal thus is not formed by the sidewall of the stopper plug pressing into the inner diameter of the vial neck. Such a seal can only be effective until the moment the vial is crimped. More on this can be found in the separate chapter of this book on container-closure integrity.

Freeze Drying Closures

Obviously these closures are not used in powder or liquid fills but in lyophilization, or freeze-dry, applications. In the lyophilization process, the drug in its liquid state is filled into the vials. The freeze-drying closure is put on the vial in a halfway down position, so that there is a vent opening between the inside of the vial and the area around the vial. Through this opening, sublimation of the liquid takes place under the influence of underpressure in the lyophilization chamber and heat that is transmitted by the plates of the freeze-dryer. At the end of the lyophilization cycle the stoppers then are fully pressed down into the vials by the shelves of the freeze-dryer (Figs. 3 and 4).

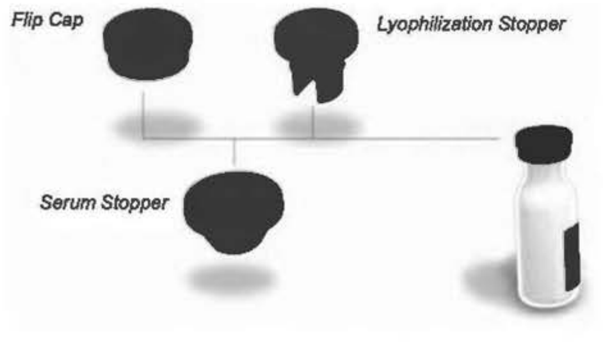

Figure 3 Various closures for serum and for freeze drying vials.

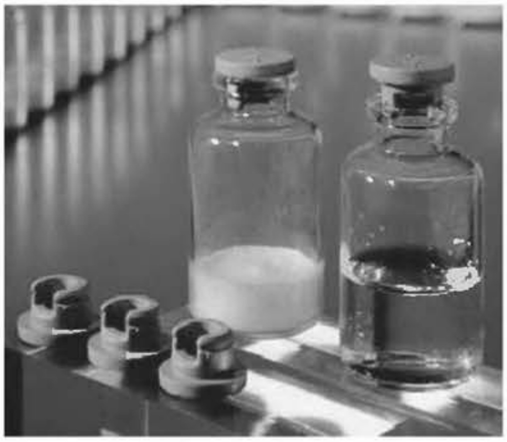

Figure 4 Lyophilization vials and their stoppers. The vial on the right hand side has a stopper in its halfway down position before freeze drying; the vial on the left has its stopper fully pressed down after freeze drying. In front of the vials are stoppers showing their lyophilization opening.

Lyophilization stoppers need to be stable in the halfway down position, to allow for proper mass transfer (sublimation!), and to prevent falling off the vials during the transport between the liquid fill station and the lyophilization chamber.

The dimensions of the closure plug, including diameter and height of the zone underneath the flange to the vent opening, must provide enough surface area to contact the vial in such a way that seal integrity is not jeopardized, from the time between unloading of the freeze-drying vials from the lyophilization chamber to the moment of crimping the vials. In practice several hours may develop between these two time points. However, if the closure dimensions are too large, then interference during initial insertion and during full insertion of the lyophilization closure may pose a problem.

Antistick markings in general are designed as part of the closure to prevent sticking/mating of stoppers during bulk transportation and within feeding lines. Another primary function of these markings with respect to lyophilization closures is to prevent closure adhesion to lyophilizer shelves upon full insertion of the stoppers. If stoppers at this stage adhere to the shelves, vials containing the freeze-dried product remain stuck to the shelves when they retract after pushing down the stoppers. This leads to undesired problems when the freeze-dryer is unloaded and to unacceptable product loss (Fig. 5).

In view of the moisture sensitivity of many freeze-dried drugs, it is clear that for lyophilization closures, penetration thickness and good control of it is of even higher importance than for serum closures.

Like serum stoppers, freeze-drying stoppers can be subdivided by their size. Most commonly found are 13- and 20-mm stoppers. Standards on freeze-drying closure design can be retrieved and ISO 8536-6 (infusion stoppers for freeze-drying) and ISO 8362-6 (infusion stoppers). Notwithstanding these standards, the market offers freeze-drying closures in a broad variety of designs, especially with respect to the design of the plug part. Each of these designs ("igloo design," "two-leg design," "three-leg design," etc.) has specific benefits in areas such as stopper stability, behavior upon reconstitution of the vial contents, and ease of withdrawal of the reconstituted from the vial (Fig. 6).

Components for Prefillable Syringes and for Cartridges

More and more drugs are packaged in prefillable syringes or cartridges, in addition to or instead of a vial presentation. Prefillable syringes are claimed to have distinct advantages over vials, including ease of use, dose accuracy and minimization of product loss in the emptied packaging.

The market offers many different presentations of prefillable syringes and it is impossible to list them all here. They consist of a series of components of various natures, but at a minimum have a barrel in glass or plastic, plus (at least) two different elastomeric sealing components.

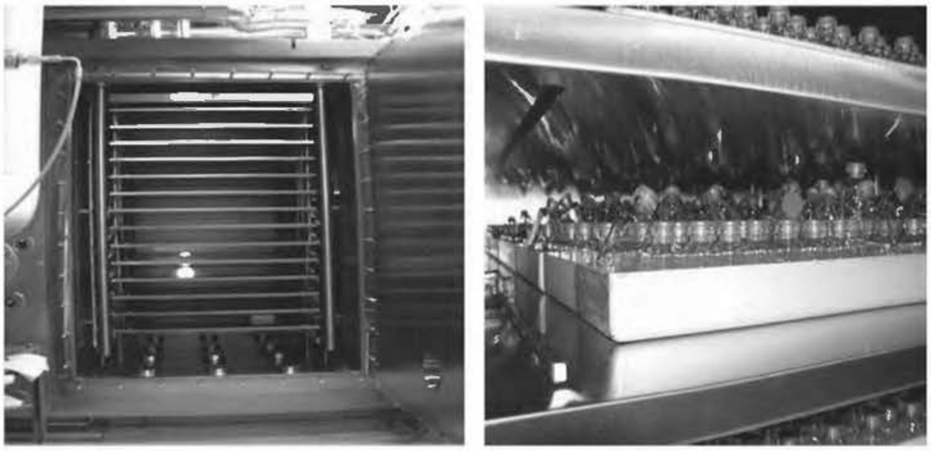

Figure 5 On the left, a picture of a pilot scale lyophilization chamber. Vials are placed on the shelves. The shelves can move so that they can bring the stoppers from their halfway down into their fully pressed down position. On the right, a picture of a shelf with vials after unsuccessful insertion of the stoppers. Stoppers got stuck to the shelf that pressed them down!

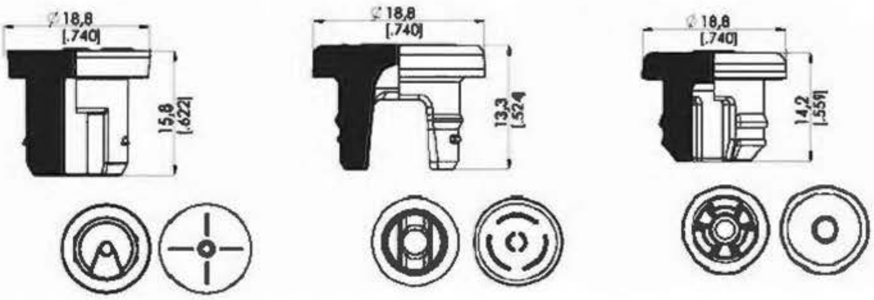

Figure 6 20 mm lyophilization stoppers in various product designs.

- An "internal" component that makes a seal on the internal diameter of the barrel. This component most commonly is called a "rubber plunger," sometimes also a "plunger stopper." After filling of the syringe this plunger is in long-term "intimate" contact with the drug, just as the cavity of the stopper plug is in case of a vial application. During the drug shelf life the plunger must maintain an adequate seal on the inner side of the barrel. However, at the time of administration of the drug to the patient, the plunger also must exhibit efficient gliding behavior in the barrel to adequately transfer the syringe contents into the patient.
- An "external" component that makes a seal between the inside of the syringe and the outer world. Basically the syringe is delivered with either a needle already being present ("staked needle") or with a prevision to place a needle at the time of administration. In the first case the needle will be protected by a rubber needle shield, also called "cover" or "sheath." The tip of the preassembled needle will stick into rubber at the interior of the needle shield, while the opening of the needle shield forms a seal on the tip of the syringe.
- In syringes without staked needle, the latter function is taken over by another rubber component, called "tip cap." The inside of the tip cap takes care of forming a seal on the tip of the syringe.

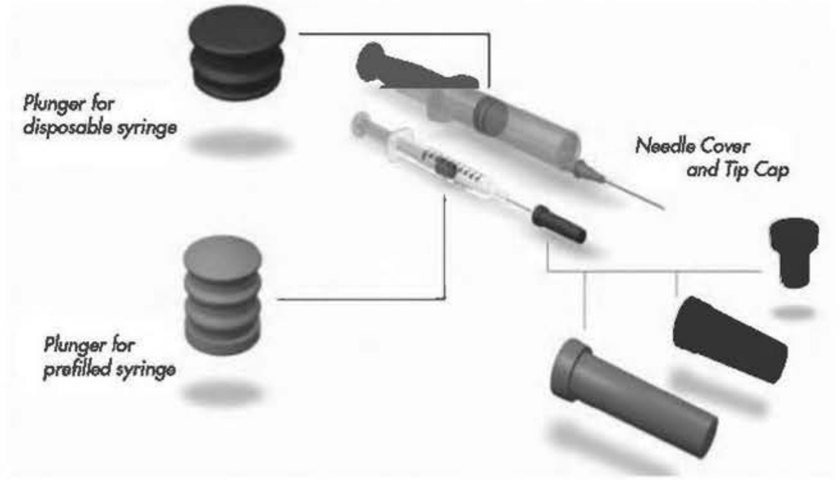

Figure 7 Elastomeric components for prefillable and for disposable syringes. Plungers on the left, needle shield and tip caps on the right.

- Even if the contact area between the syringe contents and the external rubber component may not be claimed to be zero, it is clear that this contact is less "intimate" in comparison with the contact the elastomeric plunger has (Fig. 7).

Whereas needle shields and tip caps in the past were found as components made purely out of rubber, today's tendency is to put these items into a plastic cover and, assembled in this way, to mount them onto the syringe barrel. In this case the market speaks of "rigid needle shields" and "rigid tip caps." Rigid needle shields and rigid tip caps offer or can be designed to offer enhanced product features, including tamper evidence for the syringe and extra protection against needle-stick at the time of drug administration.

Plungers for prefillable syringes are standardized by ISO 11040-5. At the time of writing there is no standard for elastomeric needle covers or tip caps.

Another prominent tendency at this time is to package drugs in cartridges. These cartridges may be intended to be used in self-administration devices, like insulin pens or growth hormone pens, or may be intended for administration by medical staff. The most well-known example in this class is a cartridge with a dental anesthetic. Like prefillable syringes, cartridges are equipped with rubber plungers. However, the second sealing element most frequently consists of a rubber disk being assembled in an aluminum cap. The cap with assembled elastomeric liner is crimped onto the front end of the cartridge. In this case, two rubber components (plunger and disk) are in long-term contact with the drug. At the time of administration the disk is perforated by a double-ended needle, one end making contact with the cartridge contents and the other end being the patient end (Fig. 8).

Typical for nondental applications, such as insulin and growth hormone cartridges, is that the cartridge contains multiple drug dosages. After administration of each dose, the rubber disk must adequately reseal so as to preserve drug sterility, and at every next dose the plunger must again smoothly move over a small distance.

Information on standardization of plungers for dental cartridges and plungers for pen systems can be found in ISO 11040-2 and ISO 13926-2, respectively.

Components for Disposable Syringes

Apart from prefillable syringes and cartridges a very large amount of rubber plungers, sometimes also called "gaskets," are used in disposable syringes that are used to administer parenteral products to patients.

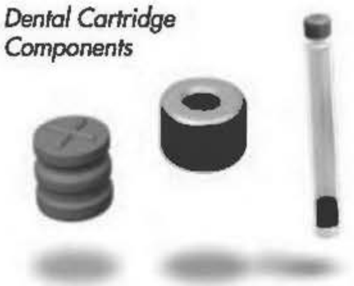

Figure 8 Dental cartridge components.

A similarity between a disposable and a prefillable syringe is that in both cases the plunger must be able to move smoothly, with a well-controlled force to start the movement and with a well-controlled force to sustain the movement as long as this is needed. A very important difference between the plungers in prefillable and in disposable syringes however is the contact time with the drug. For a prefillable syringe this time is expressed in years, whereas for a disposable syringe plunger it will be minutes or hours. This difference has a large impact on the type of material that the plunger is made of. A prefillable syringe plunger will be designed to ensure adequate gliding behavior as well as to aim for low levels of material that could be extracted from the rubber into the drug product as a leachable, while disposable syringe plungers will be designed primarily to ensure acceptable administration behavior.

Plungers for disposable syringes are standardized to some extent by ISO 7886-1.

Other Components

There are many other elastomeric components used in parenteral products, other than the ones listed so far. Among the products that are in long-term contact with parenteral drugs it is worth mentioning here parts that are used in special systems such as dual chamber syringes or vials with two compartments. In the category of short-term contact products certainly components for injection ports on flexible bags and parts used in blow-fill-seal applications take a large part.

RUBBER COMPOUNDS FOR APPLICATION IN CLOSURES FOR PARENTERALS

This part of the chapter contains information on the composition of elastomeric closures for parenterals and explains which rubber compounds are suitable in the various applications.

General Outline

The main characteristic of an elastomeric material is its elasticity. Elasticity is introduced by cross-linking the polymer chains of the elastomer base of the material by using cross-linking agents. This cross-linking process, also called "vulcanization" or "curing," uses curing agents that make chemical bonds between polymer molecules. The vulcanization takes place under the influence of temperature and pressure in a heated mold. During the vulcanization the rubber will adopt the shape of the cavities of the mold in which it is being cured. In this sense one speaks of "thermoset" rubbers.

Before vulcanization, the elastomer behaves in a plastic way, as mechanical deformation will result in a permanent deformation. By cross-linking, the elastomer turns into a rubber. After vulcanization the resulting rubber material behaves in an elastic way, and as such after imposing and taking away a mechanical deformation the material will regain its original shape.

The total set of materials that are used in rubber compounds can be listed as follows:

- *The elastomer*: It is the polymer base of the compound. A rubber compound may either use one single elastomer or a blend of different elastomers. The type of polymer(s) will heavily influence a number of characteristics of the resulting rubber.

- *The cure system*: It consists of a defined set of chemicals that take care of the cross-linking reaction. This set not only comprises the actual cross-linking agent that makes the chemical bonds, but also other chemicals that activate or accelerate the cross-linking reaction.

  There are many types of cross-linking agents of which sulfur for sure historically is the best known. Other types are phenol-formaldehyde resins, peroxides and amines. A well-known activation system is zinc stearate or zinc oxyde in combination with stearic acid. The zinc ion therefore may be readily found in aqueous extracts of quite a number of rubber materials.

  Special caution shall be given to the use of accelerators in rubber compounds for parenteral applications. In fact, these accelerators typically are organic molecules like thiurams, sulfenamides and thiazoles that are relatively easily extractable and some of which, like 2-mercaptobenzothiazole, are directly linked to health hazards, while others may give rise to the formation of hazardous reaction products as nitrosamines. Modern, unconventional curing systems for parenteral rubber compounds therefore will avoid the use of such accelerators.
- *The filler*: It attributes mechanical strength to the rubber compound. In modern parenteral applications the fillers that are used most commonly are inorganic mineral materials like aluminum silicate (clay) and magnesium silicate (talc). Carbon black, which is commonly used in other rubber applications, is avoided for use as filler for parenteral applications. This is due to the potential link with polynuclear aromatics (PNAs) that may pose a health hazard.
- *The pigment*: It attributes a color to the compound. In parenteral applications most components are gray, red, or black. The gray color is obtained by incorporating titanium oxide (white) and minor amounts of well-defined carbon blacks. The red color comes from the use of red iron oxide. Pigments for rubbers for parenteral application preferentially are not of organic nature, again because they may be extractable.
- *Other rubber ingredients*: In this class are various materials that either influence the physical properties of the rubber, like plasticizers, or the physicochemical stability of the rubber compound, like antioxidants and antiozonants, or the surface state of molded products, like migrating plasticizers or waxes. Modern parenteral rubber formulations will use these ingredients only if really needed and at any rate their extractability will be a design factor in the development of the compound.

Halobutyl Compounds

For parenteral applications, the most widely used compounds for long-term contact applications (vial stoppers and plungers for prefillable syringes and cartridges) are pure halobutyl compounds or are blended compounds where the halobutyl polymer is the main elastomer.

There are three major reasons for this. First, halobutyl elastomers allow for the lowest possible gas permeability of polymers that are available worldwide on an industrial scale. For sure in parenteral applications, where oxygen and moisture permeability are an issue, this is of the highest importance. Also, even if it cannot be linked one to one, low gas permeabilities are linked to lower absorption characteristics, especially with respect to preservatives that are present in parenteral formulations, and with lower leaching characteristics into the drug.

Secondly, halobutyl compounds allow using the cleanest curing systems. Accordingly, vulcanization can be obtained using the smallest possible set of curing agents with low extractable potential.

Thirdly, halobutyl elastomers, thanks to their low level of unsaturation, have extremely good ageing characteristics. This allows working with the lowest possible antioxydant levels, thus again preventing extractable and leachable issues, and still achieving a shelf life of multiple years.

Traditional halobutyl elastomers are obtained by polymerization of isobutylene and isoprene, followed by chlorination or bromination of the resulting copolymer. In the mid-1990s an even more stable brominated copolymer of isobutylene and para-methylstyrene was brought to the market. This new elastomer at present time is used in a small number of parenteral rubber compounds only.

It is to be noted that nonhalogenated copolymer of isobutylene and isoprene, named butyl elastomer, equally may be in use for parenteral applications. Little or no new rubber compounds based on butyl elastomer are however offered to the market anymore.

A frequently asked question is whether bromo- or chlorobutyl is to be preferred. The answer is that principally bromobutyl cross-linking can still be achieved in a "cleaner" way, however the difference with chlorobutyl cross-linking is not of practical relevance. In fact, the use of bromobutyl or chlorobutyl compounds can be linked to a historical or even geographical background. Furthermore, it is very often forgotten that it is not so much the elastomer that is responsible for the chemical cleanliness of a parenteral rubber compound, but rather the rest of the compound recipe!

Poly-isoprene Compounds

Whereas halobutyl compounds stand for impermeability, chemical cleanliness and high stability, it is difficult to achieve with these materials the levels of elasticity that are required in some parenteral applications. Notorious in this respect are multipuncture applications, as encountered for instance with stoppers on insulin vials or with rubber seals on cartridges containing insulin or growth hormone. If the number of penetrations with a needle is tens of times design specifications sometimes are over 100 times it is not possible to ensure proper functionality in the sense of adequate resealing and of absence of coring with a pure halobutyl compound. For these applications historically natural rubber compounds or blends of halobutyl and natural rubber or laminates of these two materials were used. Since the last decade of the 20th century however, natural rubber has been largely phased out for use in pharmaceutical and medical rubber since, justifiably or not, it is associated with the risk of "latex allergy." Synthetic poly-isoprene has replaced natural rubber in most applications.

While mechanically superior to halobutyl compounds, poly-isoprene compounds fall short in other areas that make halobutyls so performant for pharmaceutical applications. Oxygen and water vapor permeability of poly-isoprene compounds are one to two orders of magnitude larger than for halobutyl materials. Poly-isoprene compounds also require more complex cure systems, which often means less pure and / or higher concentrated cross-linking agents. Residuals of the cure system in a number of cases may migrate to the surface of poly-isoprene components ("blooming"). Ageing characteristics of poly-isoprene compounds need to be improved by incorporating higher levels of antioxidants and in forthcoming case by including antiozonants.

In a number of applications components made of distinct layers of a halobutyl compound and of a poly-isoprene compound are able to bring a solution that offers the best of both worlds. This type of solution can be applied in the case of seals on insulin cartridges, where the rubber disk may be a laminate consisting of halobutyl material facing the drug and with a poly-isoprene side not in contact with the drug, however ensuring perfect resealability upon multiple puncturing. Unfortunately, such a laminate solution is not industrially feasible for vial stoppers.

Other Compounds

Whereas most parenteral applications call for low permeability compounds, some do just the opposite. The most important example is that of an elastomeric needle shield for a prefillable syringe. In a lot of cases these needle shields are preassembled on the cleaned and siliconized barrels of prefillable syringes with staked-in needles, packaged in gas permeable tubs and then subjected to ethylene oxide sterilization. Since the open end of the needle shield forms a hermetic seal on the hub of the syringe, the ethylene oxide must be able to permeate through the wall of the rubber shield to have its sterilizing effect on the needle that is covered by it. The needle cover thus must have a high instead of a low gas permeability. Rubber compounds used for these needle covers, and partly also for tip caps for prefillable syringes, therefore are made of poly-isoprene compounds, or alternatively of a compound based on a styrene-butadiene elastomer [styrene-butadiene rubber (SBR)]. The latter also displays a suitable gas permeability for this application.

The use of compounds other than halobutyl, poly-isoprene and SBR on the parenteral scene is for the most part restricted to niche applications. Examples are nitrile rubber for use in combination with mineral oil based drug formulations, which is often seen in veterinary applications, and silicone rubber in ophthalmic applications.

COATED CLOSURES

The compounds for elastomeric components for long-term contact with parenterals are designed to have no or the smallest possible level of interaction with the drug. For most applications, halobutyl formulations are able to achieve this goal. However, in a number of cases requirements are so high that halobutyl compounds are not adequate. Worth mentioning in this respect are biotech drugs that are used in very small quantities per dose and where no absorption by the vial stopper is allowed. Another example is cephalosporins, which in contact with halobutyl stoppers always tend to develop a measurable level of turbidity that in a number of cases is not deemed to be acceptable.

For such applications, solutions are offered to the market in the form of coated vial stoppers and coated syringe plungers. The two products that have established an accepted market position utilize fluoropolymer coatings, at least in the contact zone with the drug. Depending on the manufacturer of these coated components, the coating may have a different level of fluorination, but always will be high. Also, in all cases the coating will exhibit barrier behavior between the rubber component and the drug. This means that leaching of materials from the stopper into the drug and from the drug into the stopper is further suppressed. This in combination with the inert nature of the fluoropolymers that are used leads to better stopper/drug compatibility.

It is important to point here to the fact that the barrier function of coatings is not absolute. While extractables and leachables will be reduced, this will not be to a level of zero. The level of extraction will in part be dependent on which extractable is involved, as to whether the barrier function of the coating will be stronger or weaker. Where fluoropolymer coatings are not barriers is against water vapor. Fluoropolymer coatings thus are not suitable for preventing uptake of moisture during steam sterilization.

A difference between the two types of coated closures in the market, apart from the identity of the fluoropolymers, is the area in which the barrier coating is applied and how it is applied.

The first type starts from a fluoropolymer film that in a special type of molding process is applied to the closure in the contact area with the drug only (the largest part of the plug for a vial stopper). Other parts of the stoppers, including the topside, sidewall and underside of the flange and the part of the plug immediately underneath the flange, are left uncoated. This allows for achieving compatibility improvement with the drug with a thicker film of fluoropolymer material. The top part of the flange of these stoppers still needs some sort of siliconization to avoid stopper clumping during transport and machining. Equally it is debatable whether the entire drug contact area is coated or not.

The second type of coated closures uses fluoropolymer that is deposited on the closures in a proprietary type of spray coating process. The coating in this case is thinner, however still clearly exhibits a barrier function. This process enables coating of the entire closure, not only in the drug contact zone but also in all other areas. Since the coating is nontacky in itself, these closures do not require any surface siliconization, which in applications where the drug is sensitive to silicone of course is of highest value. Also coating of the sidewall of the flange is of help in prevention of formation of particulates during machining of the stoppers in feeding bowls and in chutes.

Fluoropolymer coated closures are available as vial stoppers and more recently also as plungers for prefillable syringes. Coated vial stoppers may require minor adaptations to the settings of filling machine but for the rest do not require too much attention in terms of machineability. This is different for coated plungers, especially when they are strongly mechanically stressed when they are inserted into the barrels of the syringe. At this stage the coating may start to exhibit wrinkling which worst case may lead to marginal sealing behavior on the inner diameter of the syringe barrel. Precautions to prevent this are indicated, either by using a suitable filling technique or by using adapted machine parts.

Coated closures mostly are encountered in high value applications, like biotech drugs, or for silicone sensitive drugs like some proteins. Since these closures require the use of costly fluoropolymers plus the use of extra process steps to apply the coating, the cost of coated closures is considerably higher than for uncoated closures. In spite of their superior product properties this high cost precludes their more widespread use, especially in cases where the cost of the component is not negligible compared with the cost of the drug.

PROPERTIES OF PHARMACEUTICAL RUBBER AND OF CLOSURES

This part of the chapter gives an overview of the most important properties that are or can be of interest for closures for parenteral application. The overview lists both properties of the elastomeric material itself and properties of components made thereof.

Physical Properties

Hardness

Hardness is the physical property of a rubber that is most apparent to the user since manipulating the closure or penetrating it with a needle gives an idea of its hardness. The hardness of a rubber is determined by a number of factors. The most important ones are the ratio of filler to elastomer and the presence or absence of a plasticizer. For a given compound system hardness will increase with increasing the amount of filler relative to the elastomer. Hardness of closures for parenteral applications is usually in three ranges: soft, hard and intermediate. The softest formulations can be found in applications where resealing is of critical importance, such as in injection points for flexible bags. These formulations tend to have no or only a low amount of filler. Most vial stoppers on the other hand are in an intermediate range. Softer stoppers, in as far as they do not contain a plasticizer, are made of formulations with relatively little filler, while in harder stoppers the ratio of filler to elastomer is higher. The hardest formulations for parenteral applications will be found in syringe plungers. The background for that is that gliding forces for harder formulations are more favorable than for softer ones.

There are numerous scales in which hardness of materials is expressed. Hardness for rubber formulations for parenteral closures though is expressed in Shore A. Values that are encountered in practice are in a range of grossly between 30 and 55, with exceptionally numbers up to 65° to 70° Shore A.

Hardness of rubber formulations is measured according to standardized methods on test buttons of standardized dimensions. ISO 7619-1, "Rubber, vulcanized or thermoplastic Determination of indentation hardness Part 1: Durometer method (Shore hardness)" is such a method. As the title already indicates the hardness of a rubber is determined by measuring the indentation depth of a standardized "pin" into the test button. There is often confusion about the fact that the value that results in this way cannot be reproduced by measuring on the rubber product (stopper or plunger) itself. Values measured on closures therefore will often be out of the hardness range that the closure manufacturer specifies on their data sheets.

Ash Percentage

Ash percentage measures the portion of noncombustible material in a rubber compound. This comes down to measuring the portion of material of inorganic nature to material of organic nature in the rubber material. Inorganic materials in rubber compounds for parenterals are primarily fillers, and to a lesser extent the pigment and potentially a portion of the cross-linking system. Materials of organic nature in rubber compounds are of course the elastomer, and also potentially a plasticizer. Since the primary inorganic and organic constituents are filler and elastomer, respectively, and since hardness is primarily determined by the ratio of these two, it is not surprising that hardness and ash percentage are linked to each other. Basically they yield the same information about the rubber formulation. Hardness though is less laborious and less cumbersome to measure in comparison with ash percentage. A standardized method to measure ash in rubber is ISO 247, "Rubber Determination of ash."

Compression Set

Rubber is used for parenteral closures because of its elasticity, or its ability to return to its original form after being mechanically compressed. Yet, rubber is not perfectly elastic. This means amongst others that if a mechanical compression is being exerted for a long time on an elastomeric component, that it will not 100% return to its original form again. The difference between the original and the final form is called "permanent deformation." There is a standardized test (ISO 815) that measures permanent deformation of rubber under standardized conditions. It expresses the permanent deformation of a test part as a percentage of the deformation that the part was subjected to. This percentage is called "compression set."

The higher compression set of a rubber is, the higher thus is its permanent deformation under influence of a mechanical load. Expressed differently, the higher is the tendency of the rubber to adapt to the shape of its environment. Translating this into practical terms for prefillable syringe plungers that are compressed for a long time into a barrel, it means that the outer diameter of plungers made from a rubber with a high compression set tends to adapt to the inner diameter of the barrel. Of course, this is not desired or at least must be under control, since the plunger is expected to yield over time a high enough force on the inside of the barrel to guarantee seal integrity before and at the time of activation of the syringe.

Parenteral applications thus call for elastomeric materials with low enough compression sets. When measured according to ISO 815 (24 hours at 70°C) compression sets for rubbers for parenteral applications will be found to be in a large range between 10% and 50%. Depending on the application this may or may not be acceptable. A typical compression set for a halobutyl compound is in the range of 15% to 40%.

It is worth mentioning here that γ irradiation has a significant impact on the permanent deformation of rubber. This means that when rubber is subjected to the simultaneous action of mechanical compression and of γ irradiation its permanent deformation will be larger than when subjected to compression alone. The difference between the two, which is also function of the irradiation dose, can, depending on the rubber, range from significant to very significant. There are rubber formulations that have an acceptable compression set but an unacceptable "irradiation set," which means that under the combined action of compression and irradiation their permanent deformation is too large to still guarantee functionality. This aspect must be taken into consideration when making selections like that of an elastomeric part for a syringe that is irradiated with the plunger being assembled.

Gas Permeability

It has been pointed out in paragraph 3 of this chapter that gas permeability is a property of major importance for elastomeric closures used for parenterals. The majority of parenteral applications call for low permeability of the rubber closure (vial stoppers and prefillable syringe plungers), however as explained in a previous part of this chapter some applications require just the opposite (needle shields and tip caps for prefillable syringes).

The two extremes of permeability in the parenteral area are formed by halobutyl rubber (low permeability) on one hand and poly-isoprene or natural rubber (high permeability) on the other hand. In between are rubbers like SBR. Relative oxygen permeabilities at 40°C for different rubber compounds as cited by literature and confirmed by own measurements are approximately 1 for halobutyls to about 10 for SBR to 20 to 30 for poly-isoprenes. Similar relative rankings apply for moisture vapor permeability measured at the same temperature. Gas permeability of a rubber primarily depends on the type of polymer, but also on other factors as the type and degree of filler. Among external factors that influence gas permeability certainly temperature needs to be mentioned, with higher temperatures causing higher gas permeabilities.

The ISO standard to measure gas permeability is ISO 2782. For pharmaceutical rubber it is however more common to refer to ASTM standards ASTM D3985 (oxygen) and ASTM F1249 (water vapor).

It is worth mentioning here that recently instruments have been introduced into the market to nondestructively measure moisture or oxygen in the headspace of individual vials. The technique is based on laser absorption spectroscopy.

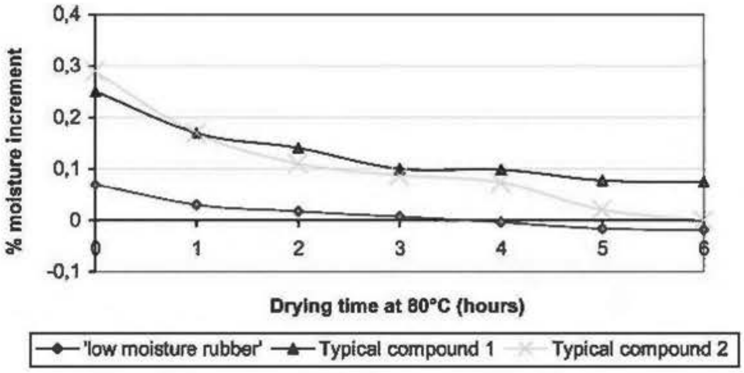

Figure 9 Moisture uptake and release of three different rubber formulations. The curves show moisture uptake at initial steam sterilization (30 minutes at 121°C) and subsequent release at drying at 80°C.

Moisture Absorption/Desorption

Water vapor permeability of the rubber compound influences the amount of water that over time will permeate through the rubber closure into a vial with medicinal product. Another factor that influences the amount of water that will permeate through the rubber closure is the amount of water that is withheld in the stopper itself at the moment it is placed on the vial. This moisture over time will partly end up in the drug product. Whereas for aqueous solutions this will not be of an issue, it can be for moisture sensitive products that are filled as powder or are freeze-dried. The lower the amount of active pharmaceutical product contained in the vial is, the more critical the situation can get. Therefore, in cases where moisture sensitivity of the drug formulation is an issue it is indicated to monitor the moisture content of the elastomeric closure at the moment of filling.

The moisture content of halobutyl stoppers in the state as they are supplied to pharmaceutical companies typically is in the range of 0.3% to 1%. It must be stressed though that by steam sterilizing the stoppers, as is usual for aseptic filling, a significant amount of extra water is "pumped" into the closures. This extra moisture needs to be dried to a level that is compatible with the moisture sensitivity of the drug application. Recently "dry" halobutyl compounds have been offered to the market, or compounds that take up significantly less water during steam sterilization while maintaining the typical drying behavior of halobutyl materials. These dry compounds target specifically lyophilization applications. Figure 9 depicts the moisture absorption/desorption behavior of such a dry compound in comparison with two "traditional" halobutyl formulations. The time point $t = 0$ represents the percentage weight increase of the stoppers as noted during a steam sterilization of 30 minutes at 121°C. The other time points represent the drying behavior at 80°C as found during laboratory drying. It should be noted that since the stoppers before autoclaving also contain moisture, negative values for the drying part of the curve are possible.

A standardized method for measuring moisture of elastomeric closures can be found in ISO 8362-5, "Injection containers for injectables and accessories Part 5: Freeze-drying closures for injection vials." The principle of the method that is outlined there is a coulometric Karl-Fisher titration of the moisture that is dried off from a part of the stopper. The advantage of this method obviously is that it specifically measures moisture. Simple weight change methods to measure moisture absorption/desorption of elastomeric closures are also frequently used.

Absorption of Preservatives

Many drug formulations are stabilized by the use of preservatives like parabens, m-cresol, or benzalkonium chloride. These preservatives are added in low concentrations, however they have a tendency of being absorbed by rubber, thus loosing their effect in the drug solution. Depending on the combination of type and concentration of preservative and type of rubber compound this absorption can be more or less pronounced. Development of new rubber compounds and development of drug formulations must take this absorption into account.

Swelling

Many drug formulations are aqueous solutions. Water to a certain extent is absorbed by the rubber closure. Where water is in contact with the closure it may cause a local discoloration, for example, a dark gray stopper may discolor to a lighter gray. This discoloration is not of any functional concern and can be reversed by drying the stopper.

In contrast with water, other drug diluents may display a higher amount of absorption into the rubber closures. They cause a clearly measurable increase in the weight and, in forthcoming cases, in the dimensions of rubber closures. In this case one speaks of "swelling" of the stopper. Swelling usually is expressed as a percentage of weight gain of the stopper.

Oils are known to make rubber swell. For example, vegetable oils over one month will typically cause a 3% to 4% weight increase in halobutyl stoppers. Usually this will not hinder the functionality of the closure. Mineral oils on the other hand will cause a much higher swelling in halobutyl stoppers and therefore are incompatible with them. In such cases either the use of special rubber formulations (nitrile rubber) or of coated closures is indicated.

Apart from the physical effect of swelling, the diluent that penetrates the closure and is absorbed there also may dissolve rubber chemicals and act as carrier for leachables into the drug solution.

Chemical Properties

Extractables According to Pharmacopeial Methods

As set out earlier, rubber compounds are composed of different materials that have been vulcanized through a curing step at elevated temperature. In contact with a drug solution some of these materials, their impurities, their reaction products or their thermal breakdown products may be extracted from the rubber closure.

A common way to make an assessment of extractables from pharmaceutical rubber is to prepare an extract of the rubber under well-defined model conditions and then, by using primarily wet chemistry methods, to measure for extractables. Such methods can be found in all major pharmacopeia, specifically in U.S. Pharmacopeia (USP) <381>, "Elastomeric Closures for Injections," in European Pharmacopeia (Pharm. Eur.) 3.2.9, "Rubber closures for containers for aqueous parenteral preparations, for powders and freeze-dried powders" and in Japanese Pharmacopeia (Pharm. Jap.) 7.03, "Rubber Closures for Aqueous Infusions." Also ISO 8871-1, "Elastomeric parts for parenterals and for devices for pharmaceutical use Part 1: Extractables in aqueous autoclavates" is such a method.

The methods for measuring extractables in USP <381> as from 2009 on are extremely close to the methods in Pharm. Eur. 3.2.9 and in ISO 8871-1.

All three aforementioned methods use water as a model solvent and extract rubber by autoclaving it for 30 minutes at 121°C in a ratio of 1 cm^2 of rubber surface area exposed per 2 mL of water. In the aqueous extract that is obtained in this way, a number of determinations are done, including measurement of acidic or alkaline substances, measurement of reducing substances, assessment of the UV absorbance spectrum of the extract, and measurement of volatile sulfides and of zinc (both are common rubber chemicals). The results of the testing have to be within certain "type I" limits or within more loosely set "type II" limits as "fallback position." The idea behind this is that rubber for parenteral applications should be as clean as possible and thus meet the type I requirements. However for rubber articles where the mechanical requirements are so high that they cannot be met by using the cleanest cross-linking systems, the less strict type II limits allow these compounds still to qualify as "pharmacopeia compliant" or "ISO compliant."

In view of the fact that the ratio of surface area of rubber per volumetric unit of water is constant, the results for chemical testing of USP <381>, Pharm. Eur. 3.2.9 and ISO 8871-1 are independent of the size of rubber product that is extracted. Pharm. Jap. 7.03 is different. It also uses water as model solvent, however it extracts rubber in a fixed ratio of 1 g of rubber per

10 mL of water. As a consequence, for smaller rubber parts that are lighter in weight, relatively more surface area will be exposed to the extraction medium. Therefore for such small parts it is relatively more difficult to comply with Pharm. Jap. 7.03. Also the list of tests in Pharm. Jap. 7.03 is quite different from the other pharmacopeia and there is only one single set of limits.

Extractables and Leachables

No doubt the most discussed topic in the area of elastomeric closures for parenterals in the last decade has been the subject of extractables and leachables.

It has become clear that whereas pharmacopeial extractable methods are able to discriminate between cleaner type I formulations and less clean type II rubber compounds, they are not appropriate to distinguish between rubber formulations that have a general low extractable profile and compounds that are especially developed to release as little as possible to drug formulations. Also pharmaceutical companies and health authorities definitely want to know more about the specific identity of species that are released by packaging materials so that appropriate toxicological assessments can be performed.

Pharmacopeial extraction methods, with the exception of the determination of zinc, are not able to offer this. Therefore, more and more they are considered as a base level of extractable documentation that must be supplemented with more and more specific information. At the time of writing there are no standardized methods yet that describe how such additional extractable data can be obtained. However, initiatives such as the Product Quality Research Institute (PQRI) Working Group on Extractables and Leachables are underway. These initiatives no doubt over time will generate standardized methods for determining extractables under model conditions in model solvents and most likely will introduce concepts of threshold values below which extractables are accepted as safe, and above which toxicological assessments will be needed. What is then still left is the task to describe and ideally standardize the way to assess compounds from packaging materials that end up in real drug products, not in model solvents, in other words: how to assess leachables, not extractables.

A far more elaborate discussion about extractables and leachables is offered in a separate chapter in volume 3 of this reference work.

Functional Properties

Container/Closure Seal Integrity

The ultimate function of a parenteral closure is that it is able to guarantee integrity of the seal that it is forming with the container on which it is placed. Only in this way it is assured that sterility of the vial contents is preserved and that label claim specifications are met. USP <1>, "Injections," in this respect states that "containers are closed or sealed in such a manner as to prevent contamination or loss of contents." For a stopper sitting on a vial, the seal, after capping of the vial, is formed between the underside of the flange of the stopper and the top part of the vial neck. For a plunger for a prefilled syringe the seal is formed between the ribs of the elastomeric plunger and the inside surface of the glass or plastic barrel. For prefilled syringe needle covers and tip caps the seal of the elastomeric part with the cone of the syringe barrel must exhibit integrity.

USP's general chapter <1207>, "Sterile Product Packaging Integrity Evaluation" discusses the maintenance of microbiological integrity of sterile product packaging over the life cycle of the medicinal product. Integrity testing should take place during three phases: product package development phase, routine manufacturing phase and marketed product stability phase.

Closure/vial seal integrity testing methods fall into two classes: microbiological methods and physical methods. Microbial methods include liquid immersion challenge tests and airborne microbial challenge tests. Under the physical methods there is a whole array including generally accepted dye ingression methods, gas leak methods, vacuum or pressure decay or retention methods, and relatively simple weight loss/weight gain methods.

Since closure/vial seal integrity is so intimately linked to microbial integrity and preservation of sterility, one would expect that standardized microbiological challenge test methods would have developed and could be found in the major pharmacopoeia and in international standards. This however is not the case. In none of the pharmacopeia are any microbial ingression test methods described in concrete wording, while in existing ISO standards all closure/vial seal integrity testing methods to date are physical methods, notably dye ingression methods.

At this place no extensive overview of closure/vial seal integrity methods will be given. An extensive discussion of the topic is given in a separate chapter of this volume. Also PDA's technical report no. 27, "Pharmaceutical Package Integrity," 1998, is a very useful review document.

Coring

Functional test methods for elastomeric closures that are well described in pharmacopeia are coring, penetration and resealing after puncturing. A description of test methods for closures intended to be pierced with a hypodermic needle is available in Pharm. Eur. 3.2.9, as well as in USP <381>. The test methods are the same as in ISO 8871-5, "Elastomeric parts for parenterals and for devices for pharmaceutical use Part 5: Functional requirements and testing."

Coring, sometimes also termed "fragmentation," is the phenomenon whereby upon puncturing a stopper, small parts of the closure are dislodged by piercing or by abrasion. These small particles risk eventually being injected into patients. The latter of course is undesired.

Looking at vial closures for SVP and hypodermic needles, coring test methods consist of piercing a fixed number of closures a fixed number of times and collecting on a filter the particles that are formed by these penetrations. The number of particles that is visible with the naked eye must not be larger than a certain limit value.

Factors that influence the result of the coring test for SVP closures are multiple. A perhaps still nonexhaustive list is the following:

- *Physical properties of the closure*: Most important in this respect are the closure's hardness and tear strength which are both linked to the closure composition. In general softer closures tend to be less prone to coring. So are closures made from elastomeric formulations with high tear strength. The link between these properties and coring results however is not unique, as there are formulations with more elevated hardness that still are acceptable in terms of coring behavior.
- *Penetration thickness of the closure*: All other things remaining the same, higher piercing thicknesses increase coring tendency.
- *Single versus multiple piercing*: Clearly multiple piercing of the same closure increases the risk for coring. For closures that are intended to be pierced a high number of times, using special rubber compound formulations may be indicated.
- *Irradiation sterilization of the closure*: With quite many elastomeric closure formulations an increase in coring is seen after γ irradiation. The increase is higher with higher irradiation dose. However, typical doses of 25 kGy for various closure formulations are enough to cause coring results to go out of compliance with compendial limits. Use of specially developed compound formulations is indicated in these cases.
- *Quality and size of the needle*: Especially the finishing of the tip and of the sharp edges of the canula and the surface state of the needle are important. Dull needle tips and sharp edges that have a rough finish increase coring. The outer surface of the needle should have an adequate finish, meaning a surface that is not too rough and that is adequately siliconized, not to cause abrasion when penetrating the stopper. Thicker needles tend to yield higher coring results.
- *Surface state of the closure*: Also the surface state of the closure must be sufficiently lubricious. This can be achieved by adequately siliconizing the closure, or, in case of totally coated closures, by taking care that the coating displays enough lubricity.

- *The way the closure is pierced*: When piercing the closure out of its target area, or when penetrating it with the canula nonperpendicular to the closure surface, or when penetrating it with too high speed, the risk of coring increases.

Of course also for LVP coring is an issue. In case the LVP is contained in a glass bottle or in a Blow-Fill-Seal package ("bottelpack") the elastomeric closure will be pierced with a spike of considerably larger outside diameter than a hypodermic needle. Spikes of this type, unlike hypodermic needles, are made out of plastic. The same list of factors influencing coring as for SVPs is valid. Coring of LVP closures that are penetrated with a plastic spike is not described in any pharmacopeia. Test methods can be found in standards ISO 8536-2, "Infusion equipment for medical use Part 2: Closures for infusion bottles" and ISO 15759, "Medical infusion equipment Plastics caps with inserted elastomeric liner for containers manufactured by the blow-fill-seal (BFS) process." These test methods use steel spikes with specified dimensions.

Penetration Force

Elastomeric closures for parenterals must have an adequate penetration force, or a force high enough to feel some resistance upon puncturing but more importantly not too high. With respect to factors influencing penetration force again the same list as above can be used, although single/multiple piercing is not relevant for this property. Penetration force testing for SVP and LVP closures is described in the same pharmacopeial paragraphs and the same ISO standards as for coring.

Typical penetration forces for SVP elastomeric closures are between 2 and 3 N.

Resealing

Resealing of an elastomeric closure concerns its ability to perfectly reseal after being punctured and after withdrawal of the needle (or in forthcoming case the spike). Resealing must be guaranteed to preserve sterility of the vial contents before the next penetration of the closure. It is clear that resealing is only relevant for closures that are intended to be pierced more than once. Resealing of elastomeric vial stoppers for SVP's again is described in the same pharmacopeia and standards as where coring and penetration force are described. The type of test method that is found in standards always is a physical dye ingress method. A number of penetrations equal to 10 is assumed. In practice, for some drug products the number of penetrations can still be higher. In the development stage of such products this must be taken into account. SVP stoppers that are crimped on vials are pierced 10 times. Thereafter the vials + stoppers are put in a dye bath where they are subjected to an underpressure for a certain time. After atmospheric pressure has been restored it is observed that no dye has ingressed through the stopper area where the multiple piercing took place.

Applications where the number of penetrations definitely is higher than 10 are cartridges, an example of which is those that contain insulin or human growth hormone. Such cartridges are intended to be used in pen systems for self-administration by the patient. They consist of a glass barrel that is sealed at one end by a rubber plunger and at the other end is crimped with an aluminum cap containing an elastomeric liner of thickness 1.5 to 2 mm typically. At every activation of the pen system a new double-ended needle is to be used. One end of the needle penetrates the rubber liner, the other end penetrates the patient's skin. The number of activations for such pen cartridges may go up to 50 or more times. In the development stage of such products a safety factor concerning number of penetrations is taken into account, as even if the cartridge is developed to contain 50 doses, testing of resealing during system development will take place at two to even three times this number of penetrations. A perfect reseal of the elastomeric liner is difficult to realize. Substantial improvement can be achieved by using a laminate liner, or a liner that consists of two layers of nonidentical elastomeric formulations. The layer that is not in contact with the drug is made of a formulation that is specially developed with a view to multiple piercing and perfect reseal while the layer in contact with the drug is made of a cleaner rubber formulation. In practice the layer that promotes resealing (and at the same time also improves the coring behavior of the seal) will be a poly-isoprene formulation, while the contact layer with the drug will be a halobutyl formulation.

Spike Retention Force

LVP closures are pierced with a spike. This spike is part of an infusion set that makes the connection between the contents of the LVP package and the patient. The spike will be sitting in the closure for the entire duration of the administration of the LVP to the patient. Since the LVP package itself during administration will be hung up, the spike will be remaining in a hanging position in the closure for potentially several hours. During this time the closure should exert sufficient force on the spike, so that it does not slip out of its position, also not when the patient is transported between different locations in the hospital. This force is called retention force.

Retention force testing may take place in two ways, a static way and a dynamic way. In the static testing mode a well-known weight is attached to the spike for a well-known time. During this time the spike shall not slip out of the closure, nor shall any leakage of liquid be observed in the seal area between the spike and the closure. In the dynamic testing mode the force needed to pull the spike out of the closure is measured on a force testing machine.

Methods for testing spike retention can be found in ISO 8536-2 and in ISO 15759, both of which were previously mentioned in the paragraph "coring."

Gliding Behavior

Vial stoppers take care of closure/vial seal integrity during the shelf life of the medicinal product and play their functional role when at the time of administration they are pierced with a needle or a spike. Syringe plungers partly have a different functionality. Clearly they assure closure/vial seal integrity, but obviously they are not pierced. Instead at the time of administration to the patient of the drug in the syringe they must be able to assure a smooth gliding in the syringe barrel.

When looking at the gliding behavior of syringe plungers one makes distinction between the force that is needed to make the plunger start moving and the force that is needed to sustain movement of the plunger. The former is typically called "activation force" or "break-loose force," while for the latter the names "gliding force" or "extrusion force" or "propagation force" are used.

A typical force curve for the gliding of a plunger in a prefilled syringe is given below. The curve displays the force that is needed to move the plunger as a function of the distance that the plunger travels into the syringe barrel. From this curve it follows that it needs a certain build-up of force to start the movement of the plunger. Thereafter the force to keep the plunger moving decreases. Gliding forces thus are typically lower than break-loose forces. Break-loose forces must be low enough to guarantee smooth activation of the syringe. Gliding forces equally must be at an acceptably low level. Moreover gliding forces must be continuous, or without increases and decreases. Should the movement be "interrupted," then one speaks of shattering of the syringe. Shattering obviously for the comfort of the patient must be avoided (Fig. 10).

There are many factors that have an impact on gliding behavior of plungers in a syringe. One variable for sure is the design of the plunger. Forces are higher the more surface area of the rubber part is in contact with the inside of the barrel. The number of sealing ribs of the plunger and the way they are dimensioned thus play a role. Next there are the physical properties of the plunger. Harder plunger materials tend to yield lower gliding forces. Also the barrel material has an impact. Glass and plastic barrels of the same dimensions will give rise to different gliding behavior of the same plungers. Furthermore there is the surface state of the elastomeric plunger and of the inside of the barrel. This surface apart from exceptional cases is always siliconized. The degree and way of siliconization of the plunger, the degree and way of siliconization of the inside of the barrel and the homogeneity of siliconization of the inside of the barrel over the total path length of the plunger strongly influence break-loose and gliding forces. More sophisticated application methods that guarantee better homogeneity of silicone distribution in barrels as well as methods to verify this distribution recently have emerged.

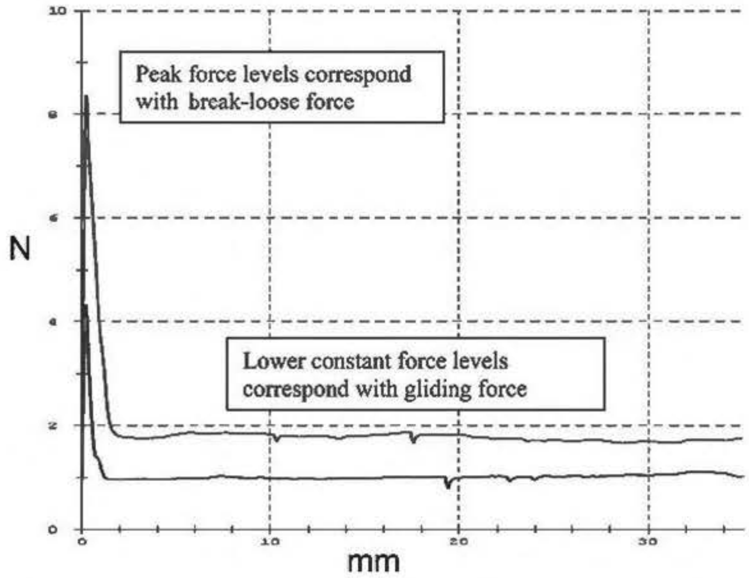

Figure 10 Gliding curves of two different plungers in the same type of barrel. The curves display gliding force as a function of the pathway of the plunger. At the left hand side, peaks correspond with break loose (or activation force). The lower part of the curves corresponds with the gliding force for the two different plungers.

Biological Properties

In this paragraph the biological properties of materials for elastomeric closures are discussed. Discussion of the state of biological cleanliness of elastomeric closures themselves in terms of presence/absence of endotoxins and colony-forming units will take place in the next chapter.

The leading reference about biological properties of elastomeric closure materials is USP. USP <1031>, "The Biocompatibility of Materials Used in Drug Containers," spends a separate paragraph on elastomeric closures. There it is stipulated that the biocompatibility of an elastomeric material is evaluated according to a two-stage testing protocol specified in section "Biological Test Procedures" of USP <381>. Unlike plastics thus no class I-VI designations are assigned to elastomeric materials.

USP <381>, "Elastomeric Closures for Injections" in turn refers to USP <87>, "Biological Reactivity Tests, In Vitro" as the first-stage test to be performed. The tests in USP <87> are designed to measure the response of mammalian cells to specific extracts prepared from the closure material. If the requirements of USP <87> are met, then no further testing is required. If however the elastomeric material does not meet the requirements of the first-stage testing as per USP <87>, then it may still qualify as a biocompatible material by passing the "more forgiving" second-stage testing as per USP <88>, "Biological Reactivity Tests, In Vivo." USP <88> tests are designed to measure the response of animals to the injection of specific extracts prepared from the elastomeric material under test. Unlike the situation with chemical properties of elastomeric closures no class or type distinction is made between elastomeric materials that meet the requirements of first-stage testing and those that qualify as biocompatible meeting the second-stage requirements only.

USP <87> lists three possible test methods: the agar diffusion test, the direct contact test, and the elution test. In practice however it is always the Elution Test that is carried out.

USP <88> equally lists three possible test methods: the systemic injection test, the intracutaneous test, and the implantation test. Since the latter is not of relevance to elastomeric closures only the first two are carried out in practice.

Not meeting the requirements of USP <87> but still passing USP <88> is typical for elastomeric materials that use certain rubber chemicals, notably accelerators, that have a cytotoxic effect on mammalian cells as per the test conditions of the "Elution Test" in USP <87>.

The relevant ISO standard on biological material properties of elastomeric closures is ISO 8871-4, "Elastomeric parts for parenterals and for devices for pharmaceutical use Part 4: Biological requirements and test methods." In essence however this is a copy of what is described in USP. At some places ISO 8871-4 refers to the ISO 10993 series of standards, "Biological evaluation of medical devices." Also this reference however does not preclude that ISO 8871-4 and USP come to the same result regarding biological properties of elastomeric closure materials.

Compatibility Behavior

The term compatibility behavior in the case of an elastomeric closure refers to its capability to preserve identity, strength, purity and stability of the drug product that it is in contact with. A closure that is compatible thus will not interact with the dosage form in such a way as to cause unacceptable changes in the quality of either the dosage form or the closure itself, an example of which would be by an unacceptable degree of swelling.

FDA's 1999 Guidance for Industry, "Container Closure Systems for Packaging Human Drugs and Biologics Chemistry, Manufacturing and Controls Documentation" is the most prominent document that further discusses the subject of compatibility of primary packaging components including elastomeric closures with pharmaceutical dosage forms. This document, amongst others, lists examples of interactions, such as "loss of potency due to absorption or adsorption of the active drug substance, or degradation of the active drug substance induced by a chemical entity leached from a packaging component; reduction in the concentration of an excipient due to absorption, adsorption or leachable-induced degradation; precipitation; changes in drug product pH; discoloration of either the dosage form or the packaging component; or increase in brittleness of the packaging component."

Investigating compatibility of the elastomeric closure with the dosage form is the responsibility of the pharmaceutical company that is qualifying the closure. Changes noted during pre or postapproval stability studies thus shall be adequately addressed.

Ageing Behavior

The ageing behavior of an elastomeric closure refers to the evolution of the property profile of that closure over time. Closures that are affected by ageing will show a deterioration of some of their properties over time. By adequate studies it must be assured that this deterioration is not in conflict with the shelf life of the dosage form that uses that particular closure.

When ageing has an effect on an elastomeric closure, then that will most likely be seen in either the surface properties or the functional properties of the closure.

In terms of surface properties various effects are possible. One of those effects is that over time ingredients of the rubber migrate to the surface and form a layer there that is different in composition compared with the bulk of the article. The phenomenon is also known as "blooming." Blooming ingredients typically are low molecular weight ingredients like accelerators, oils and waxes, and fatty acids and their salts, like zinc stearate. Blooming will have an effect on the chemical properties of the closure. Blooming clearly can only occur with rubber formulations that contain certain rubber ingredients. Avoidance of these ingredients is indicated. If this is not possible, then only storage under well-controlled conditions can help to suppress surface migration.

Another ageing effect is the change of the skin of the elastomeric closure as a result of the attack of oxygen or of ozone. Particularly ozone attack is able to induce cracks at the surface of some rubber formulations. Those cracks however may penetrate further into the body of the elastomeric part, especially in components that are mechanically stressed when they are in use. Cases have been reported of ozone cracks in tip caps for prefilled syringes that resulted in splits of the entire sidewall of the tip cap. Consequently the integrity of the seal of the cap on the tip of the syringe barrel was at stake. Ageing as a result of oxygen or ozone attack is typical for particular elastomeric formulations based on natural rubber, poly-isoprene rubber and SBR that have not been adequately formulated, or those that do not contain enough antioxydant and antiozonant of the correct type. With halobutyl formulations in general there is no issue with neither oxidation nor ozone attack.

Still another ageing effect involving the surface of the elastomeric component has to do with surface siliconization. Surface siliconization of elastomeric parts is necessary to prevent clumping of the parts during storage and transport before use and to enable processing of the parts on filling or assembly lines. Surface silicone however, depending on the type of silicone and on the type of the rubber formulation, over time can be absorbed by the closure. Hereby the silicone becomes inactive at the surface. Stickiness, clumping and in the worst of cases deformation of the parts will develop. Absorption of silicone can be countered by choosing higher molecular weight silicones or by choosing silicones that are able to crosslink and so increase in molecular weight. Silicone absorption will take place earlier in rubber formulations with high permeability such as poly-isoprene. Again, in halobutyl formulations, depending on the molecular weight of the silicone, adsorption will not be or at least will be less of an issue.

Finally, also functional properties of elastomeric closures may be affected by ageing. Particularly coring, sealing and resealing behavior are to be mentioned in this respect. Again, in halobutyls, worsening of these properties over time at most is a slow process. Yet it is indicated to check as closures before they are assembled on vials may already have some age practice shows that this can go up to two to three years and to this the shelf life of the pharmaceutical product still has to be added.

At present there is no standard that is dedicated to ageing of pharmaceutical rubber parts. General guidance is given by ISO 2230, "Rubber products Guidelines for storage." For halobutyl products, at least when stored under appropriate conditions of light and temperature, an indicative shelf life of seven years is given. For poly-isoprene articles this is less. Indicative shelf lives for such articles are three to five years.

Machineability

Machineability of elastomeric closures refers to the processes at pharmaceutical or at medical device companies that are used to bring closures into their final position on vials or in syringes or cartridges. Therefore machines will be used that are designed to have a certain capacity. Such machines typically involve feeding bowls in which the elastomeric parts, mostly after sterilization, are brought in, then feeding lines or chutes that bring the closure in the vicinity of the vial or syringe and next a pick-up and positioning mechanism that assembles individual closures onto or into individual vials or syringes.

A first prerequisite is that elastomeric parts do not clump when they are brought into a feeding bowl. Clumping is very typical for halobutyl components. Clumping behavior can largely be prevented by giving an appropriate surface state to the closures. For nonpolymer coated closures this means that the surface of the closure must be designed so as to maximally prevent sticking of individual parts by including antisticking dots or bars, that the surface of the closure has an adequate roughness that is "copied" from the roughness of the mold out of which it is produced, and that the closures have an adequate degree of surface siliconization. Furthermore care shall be taken so that closures are put into their transport packaging when they are at or close to room temperature, that they are not packed too tightly and that their shelf life for storage is taken into consideration.

Feeding behavior of closures in feeder bowls and chutes mostly is a matter of adequate surface states and of adequate dimensioning of closures and machine parts, however minute details in design may have an unexpected impact here.

Insertion behavior of stoppers into vials and of plungers into syringes or cartridges also primarily is a matter of assuring the dimensions and the surface state of the closures, vials, syringes and machine parts are well adapted to each other and are well controlled.

CLOSURE WASHING AND SILICONIZATION

Elastomeric closures for parenterals are manufactured under industrial circumstances with still a lot of manual operator intervention and using industrially available materials. Closure manufacturers spend a great deal of effort to improve the cleanliness of their plants and to tighten their procedures and quality systems so as to guarantee the quality and the cleanliness of their products. Yet, unlike with plastic products, it is not possible to collect at the end of the molding and die-trimming process the resulting products and to pack them without first subjecting them to a washing process.

There are several reasons for this.

1. Before washing, the products are not in a controlled state of cleanliness. After molding most closures are die-trimmed. Silicone in some form is used as a die-cutting agent that prevents the trimming die from getting dull. This silicone, together with the whole manufacturing history of the closures that precedes die-trimming, brings the closures in an undefined state of particulate and microbiological cleanliness. Washing of the closures is necessary to bring the closures within clear specifications, therefore to bring them in a certifiable state of cleanliness, both from the point of view of microbiological and of particulate cleanliness.
2. Closures have not been subjected to a depyrogenation process as required by regulations. FDA's 2004 "Guidance for Industry Sterile Drug Products Produced by Aseptic Processing Current Good Manufacturing Practice" states that "containers and closures shall be rendered sterile and, for parenteral drug products, nonpyrogenic." Nonpyrogenicity is obtained by subjecting the closures to well-defined washing, rinsing and drying processes. More and more this washing is delegated to the closure manufacturer who therefore needs to develop validated washing programs.
3. If not siliconized, closures will clump and machineability cannot be guaranteed. As indicated in the previous chapter uncoated closures need siliconization in order not to develop clumping during storage and to be machineable on filling or assembling lines. Closure siliconization typically is combined with the final washing and drying at the closure manufacturer.

Washing Procedures for Elastomeric Closures

The washing of elastomeric closures can be performed in different types of washing machines. Most often encountered are machines of the rotating drum type and, alternatively, machines that are based on an "overflow" principle. The former ones consist of a rotating drum with a perforated wall through which contamination can be removed. It is necessary for the machine to supply water of different types and the necessary auxiliaries, including silicone in forthcoming case. The drum can be partitioned or not, as it can consist of a number of smaller segments that each contain a smaller number of products. Washing and drying either take place in the same machine, or the washer is combined with the necessary dryers, equally of the rotating drum type. In overflow machines the flow of water is from the bottom of the machine through the stopper bed to the overflow. The closures are in a kind of fluidized bed state and contamination is continuously removed via the overflow. In some machines of both types apart from washing and drying also steam sterilization of the closures can be performed.

Washing programs for elastomeric closures vary from company to company, irrespective of whether it concerns a pharmaceutical company that still washes the closures or the closure manufacturer. A typical washing and drying program of elastomeric closures consists of the following steps:

- A washing step with water of a specified grade plus a detergent.
- A number of rinsing steps with water of specified grades. One of the rinsing steps may be combined with siliconization of the closures.
- A drying step with hot filtered air.

As to the types of water used for the washing of elastomeric closures it is worth pointing to two documents. The first of these documents is the 2004 FDA Guidance for Industry that was cited already earlier. This guidance mentions that "at minimum the initial rinses for the washing process should employ at least Purified Water, USP, of minimal endotoxin content, followed by final rinse(s) with WFI (water for injection) for parenteral products." The second document is the European Agency for the Evaluation of Medicinal Products (EMA)'s 2002 "Note for Guidance on Quality of Water for Pharmaceutical Use." For closures that are used for sterile parenterals this document equally speaks of purified water for initial rinses and water for injection for the final rinse. The major closure manufacturers therefore have invested in water plants and control systems for these plants so that they are able to guarantee the quality of the water that is used in the various stages of closure washing. What they have also invested in is the installation of clean rooms in which the washing and final packing of closures is performed and in developing monitoring schemes to demonstrate that these rooms are in compliance with standards for biological and particulate cleanliness.

Microbiological Cleanliness

The microbiological state of cleanliness of elastomeric closures relates to the presence or absence of microbiological contamination at their surface. This contamination may be present either in the form of bioburden that can be expressed as colony-forming units, and/or as endotoxins, expressed as endotoxin units.

Bioburden

In the majority of cases closure manufacturers do not sell their product sterile (or even "sterilized"). Alternatively, they sell their products with a defined state of high microbiological cleanliness, or low bioburden levels. This is particularly the case when closures are not rewashed at the pharmaceutical company itself. Closures in case of aseptic manufacturing at the pharmaceutical company then are rendered sterile prior to filling, mostly by steam sterilization.

Bioburden on elastomeric closures can be determined with a method as described in ISO 8871-4, "Elastomeric parts for parenterals and for devices for pharmaceutical use Part 4: Biological requirements and test methods." Such method consists of an "extraction" or "rinsing" phase where bioburden is transferred from the stopper surface to the extracting liquid, followed by determination of the number of colony-forming units in the rinsing liquid. The latter typically is done by filtration on a filter with a suitable growth medium and incubation of the filter. From the result the number of colony-forming units per square centimeter of stopper surface area or per stopper then can be calculated. Methods for bioburden determination on elastomeric closures need to be validated.

Endotoxins

In case of elastomeric closures the absence of bacterial endotoxins is taken as a synonym for the absence of pyrogenic components. As with bioburden closure manufacturers will sell their product with a defined state of endotoxin cleanliness. Determination of endotoxins equally is described in ISO 8871-4. Methods are similar to bioburden determination methods in that they consist of an extraction or rinsing step, followed by a determination step. Current practice is that most often determination is performed using an instrumental LAL method, or alternatively the LAL gel clot method. Also methods for endotoxin determination on elastomeric closures need to be validated.

Particulate Cleanliness

Elastomeric closures like vial stoppers and prefilled syringe plungers are part of a packaging system for injectables. Injectables are subjected to requirements on the presence/absence of particulate matter, including USP <788>, "Particulate Matter in Injections." Elastomeric closures thus are linked, be it indirectly, to the particulate cleanliness of parenteral products.

Particulate cleanliness of elastomeric closures can be approached from various sides. As explained, rubbers are composed of various raw materials that are mixed. If mixing is not perfectly homogeneous this may lead to imperfect dispersion of ingredients like fillers or pigments. This may be visible by a trained eye or under magnification as small particulates of ingredients like filler particles that are different in color from the rest of the stopper. These particles however are still firmly embedded in the rubber matrix and they will not be dislodged from this matrix. Thus they will never compromise particulate cleanliness of the parenteral product.

For particulate contamination that is present at the stopper surface in loose form this is different. These particles effectively may be transferred from the closure into the medicinal product without particular effort. Particulate contamination on elastomeric closures may still have the same material identity as the closure itself, may be part of the ingredients of that closure formulation (endogeneous particles), or may be contamination from the manufacturing environment that either has not been removed by washing or that is the effect of or a recontamination after washing (exogeneous particles).

USP <788> refers to microscopic methods and to light obscuration methods for the determination of particulate contamination in injections. For the determination of the particulate state of cleanliness of stoppers methods of the same types are standardized in ISO 8871-3, "Elastomeric parts for parenterals and for devices for pharmaceutical use Part 3: Determination of released-particle count." The methods consist again of two steps. In the first step the particulate contamination is transferred from the stopper surface into an extraction or rinsing liquid and in a second step the contamination that is transferred is sized and counted. For subvisible particulates a light obscuration technique is used. Particles typically are sized in classes 2 to 10 μm, 10 to 25 μm, and >25 μm. For visible particulate contamination particles are collected on a membrane filter where they are sized and counted, either by an operator or by a microscope that is connected to a suitable software system for sizing and counting of particles. Visible particles are typically sized in classes 25 to 50 μm, 50 to 100 μm, and >100 μm.

At present time there are no limit values for subvisible or visible particulate contamination of elastomeric closures, neither in any pharmacopeia, nor in the aforementioned ISO 8871-3. Limit values may be present in quality agreements between manufacturer and customer, but this is on a voluntary basis. The same holds for biological cleanliness of closures.

In case limit values for particulate cleanliness are agreed on, it must be assured that determinations at the closure manufacturer and at the user yield sufficiently comparable results. Although it seems logical that a determination method yields a result with a certain precision and accuracy, intralaboratory repeatability and interlaboratory comparability of particulate cleanliness determinations on elastomeric closures is known to be poor in comparison with other analytical methods.

Closure Siliconization

The purpose of closure siliconization has been explained before. Siliconization of closures usually is part of the final washing of the parts. In one of the rinsing steps silicone is added to the rinsing water. Closures pick up some of the silicone. The water that at the same time is picked up is removed in the drying step of the washing/drying program.

There are various types of silicone that are used for closure siliconization and there are various ways to introduce these silicones into the closure washing machine. Silicone (polydimethylsiloxane) may be introduced as pure silicone or as a silicone emulsion that makes uses of an emulsifier to hold the silicone in an emulsion. The former method is preferred since the emulsifier is not removed by drying. This means that it stays on the closure and, in case of renewed contact with an aqueous medium, as often is the case with a drug product in a vial or a syringe, it will bring the silicone in emulsion again. This emulsified silicone is detectable as subvisible particulate matter. Silicone thus acts as an important source of particulate matter in parenteral products. Also in case no emulsifier is used it deserves attention to bind the silicone as well as possible to the rubber surface. A way to achieve this is to use silicone of higher viscosity, or of higher molecular weight. The longer polydimethylsiloxane chains have lower mobility and attach better to the stopper surface. An alternative way to immobilize silicone at the closure surface is to use a crosslinkable silicone. Such silicone typically is not added in the washing stage of the stoppers but in an earlier stage when the stoppers have not yet been die-trimmed from the sheets in which they are molded. Crosslinkable silicone may be sprayed on the sheets that subsequently are subjected to a silicone curing reaction.

Silicones used for siliconization of elastomeric closures are subjected to the requirements of the USP chapter "Dimethicon" and to Pharm. Eur. 3.8.1, "Silicone used as a lubricant." The viscosity ranges of silicone in these two documents do not perfectly match. The lower limit for Dimethicon is 350 cSt (centistokes) while the lower limit as per Pharm. Eur. is 1000 cSt.

Validation of Stopper Washing

FDA's 2004 Guidance for Industry "Sterile Drug Products Produced by Aseptic Processing cGMP" mentions that "containers and closures should be rendered sterile and, for parenteral drug products, nonpyrogenic" and that "the validation study for such a process should be adequate to demonstrate its ability to render materials sterile and nonpyrogenic." For pharmaceutical companies who wash elastomeric closures themselves and then sterilize them, this implies that they develop validation programs for closure washing and sterilization. At many occasions however it is closure manufacturers who perform the last washing of elastomeric closures. In this situation, closures are not rewashed by the pharmaceutical end-users, and only the sterilization is taken care of by them. This practice implies that the depyrogenation process of the closures is delegated to the closure manufacturer who consequently must avail of a validation package for their washing program. The core of such validation studies is inspired by the statement in the Guidance that "the adequacy of the depyrogenation process can be assessed by spiking containers and closures with known quantities of endotoxin, followed by measuring endotoxin content after depyrogenation.... Validation study data should demonstrate that the process reduces the endotoxin content by at least 99.9% (3 logs)." The closure manufacturers will therefore have to develop rationales for the closures to be included in their studies so as to bracket the relevant product portfolio and for which (worst case) conditions are going to be adopted in validation experiments. Not all closures are equally easy to wash. It is accepted in the industry that the ease with which endotoxin can be removed from closures is related to the ease with which the washing and rinsing water have access to the concave parts ("cavities") of the closures. Endotoxin spiking thus for validation purposes shall be done at these parts of the stoppers. For larger stoppers with shallow cavities it will prove to be easier to demonstrate a log 3 endotoxin reduction than for smaller closures with deeper cavities.

Validation of closure washing, apart from the essential part of endotoxin reduction, will also contain validation data about the microbiological cleanliness of the parts after the depyrogenation process. Other properties such as reduction of particulate burden by washing, particulate cleanliness of washed and dried parts, siliconization and presence/absence of washing detergent may form part of washing validation, also when not required by the aforementioned Guidance.

STERILIZATION OF PARENTERAL CLOSURES

Sterilization of parenteral closures may take different forms. The contact area of the parenteral closure with the drug product must be sterile at the time of use. This is achieved by either terminal sterilization of the packaged drug or by aseptic filling where all packaging materials are sterilized prior to filling. In case of plungers for disposable syringes sterilization takes place on the assembled and packaged syringe.

Steam Sterilization

The most common method to sterilize closures for parenteral applications is by steam sterilization, either prior to aseptic filling or by terminal sterilization whereby the packaging components are already assembled. The most typical sterilization temperature that is used for sterilization of elastomeric closures is 121°C, the most typical length of the cycle is 30 minutes. Only in seldom cases higher steam sterilization temperatures such as 134°C are used. For some applications such as blow-fill-seal packages lower temperatures of 106°C or 110°C are applied. Of course every sterilization process of packaging components shall be validated.

As mentioned before steam sterilization puts a considerable amount of moisture into elastomeric closures. Therefore closures after steam sterilization shall be dried again using appropriate procedures that take into account the sensitivity of the drug product to residual moisture in the closure. Closures for lyophilization applications therefore often will be dried to lower residual moisture than closures for liquid fills. Typical drying temperatures for elastomeric closures range from 80°C to 110°C. In a number of cases drying times of only one hour are applied, in other cases drying cycles of up to sixteen hours are qualified.

Other than the moisture uptake, steam sterilization of elastomeric closures, followed by drying, will not affect their functional properties. This still holds when the cycle is applied more than one time on the closures, albeit that this shall not be encouraged and that for multiple sterilizations a check on closure functionality may be indicated, depending on the exact use of the closure in question.

Notes

1. Whereas drying at temperatures of 80°C to 110°C will not affect elastomeric closure functionality, the same does not hold for substantially higher dry heat temperatures. Depending on the elastomeric formulation in question dry heat treatments where closures are exposed to temperatures of approximately 150°C or higher for longer times (15, 30, . . . min) are to be avoided. Dry heat sterilization of elastomeric closures is to be totally advised again.
2. It is worth mentioning here that steam sterilization obviously has a sterilizing effect on elastomeric closures, however it cannot serve as depyrogenation process.

Sterilization by Irradiation

Of increasing importance is the use of irradiation sterilization for elastomeric closures. In such cases the pharmaceutical user will choose to be supplied with closures that have been washed by the closure manufacturer and that then have been subjected to a γ irradiation treatment at a sterilization contractor (see also later under "Packaging Ready to Use").

Sterilization by β irradiation of elastomeric closures is not excluded, however γ irradiation because of its much higher penetration capability is preferred. γ Sterilization of elastomeric closures can take place on entire pallets with closures packed in cartons, but more often is carried out with a more limited number of cartons, typically six or eight, being put together in sterilization "totes." One of the advantages of tote sterilization is that the dose distribution over the different cartons with closures will be more homogeneous, as the ratio of maximum to minimum dose achieved over the entire tote is smaller than the same ratio in an irradiated pallet. Since in case of irradiation sterilization the objective is to reach a validated minimum dose, the maximum dose in the case of tote sterilization therefore will be smaller compared with the case of pallet sterilization.

This is of significant importance, since unlike steam sterilization, γ irradiation is more likely to have an effect on the functional properties of the closures. γ Irradiation may have different effects in elastomeric closures. Depending on the formulation of some rubbers, additional cross-linking may take place. In others just the opposite occurs, or the rubber is de-crosslinked to a certain extent. Because of these effects in some rubbers loss of elasticity is found, resulting in a certain "hardening," "stiffening," increase in coring rate and, worst case, inadequate resealing behavior. In other cases closures after γ irradiation exhibit increased tackiness. All of these effects are more pronounced with increasing γ dose. For every individual application it shall therefore be investigated whether the applied irradiation dose does not affect the closure performance up to a level that it is no longer compatible with the requirements of the application. Especially attention has to be given to multidose applications where the closure by the nature of the application is penetrated multiple times. If there is an effect of γ irradiation on the functional properties of elastomeric closures, it will be noticed immediately after the irradiation, unlike with plastics where the effect may be delayed and become apparent only longer time after irradiation.

The most encountered γ dose applied for elastomeric closure sterilization in the past was 25 kGy. As a result of the publication of ISO 11137 on radiation sterilization of health care products newer applications use lower doses that are friendlier to elastomeric components. Of course such lower doses must be demonstrated to be efficient, therefore capable of guaranteeing a certain sterility assurance level. Information and instructions on how to achieve this are given in the same standard.

The effect of γ irradiation is most prominent with respect to the mechanical and functional properties of elastomeric closures. The effect on chemical properties is less evident. On the level of pharmacopeial compliance no effects will be noticed that would turn a compliant elastomeric formulation into a noncompliant one. On a more detailed level of extractables effects are not excluded, certainly not at higher irradiation doses.

Ethylene Oxyde Sterilization

Ethylene oxyde sterilization is very commonly used for the sterilization of disposable medical devices. In the area of elastomeric components for parenteral closures the most important case is the sterilization of disposable syringes that very often contain an elastomeric rubber plunger. Sterilization is achieved by the action of ethylene oxyde gas on the biocontamination that is present on the plunger surface. To make this action possible the syringes will be packed in gas permeable packing that allows the gas to enter into the syringe. It is well known that ethylene oxyde sterilization leaves chemical residues in the form of residual ethylene oxyde and of ethylene chlorohydrine. Suitable aeration times that allow these residues to decrease below certain levels that are considered as safe must be established.

Apart from disposable devices ethylene oxyde sterilization is very common in one other application in the parenteral field, namely in the sterilization of assemblies of needle covers and tip caps on the barrels of prefillable syringes. One way to come to a presentation of a drug in a prefilled syringe is that pharmaceutical companies purchase syringe barrels with needle covers of tip caps already assembled on them at syringe system manufacturers. The system manufacturer performs the assembly of needle covers on syringes with a staked needle or of tip caps on syringes without needles. The assemblies are then put into tubs that carry a gas permeable plastic film. The tubs next are subjected to ethylene oxyde sterilization. In the case of needle covers the ethylene oxyde has to permeate through the wall of the needle cover to reach the needle surface where the ethylene oxyde has its sterilizing effect. Also these processes of course include suitable aeration or "degassing" cycles. The sterilized barrels may then be directly aseptically filled by the pharmaceutical company and subsequently stoppered with elastomeric plungers that are sterilized prior to aseptic filling.

PACKAGING FOR ELASTOMERIC CLOSURES

The last step in the manufacturing of elastomeric closures is a packaging step. The packaging for closures may just be a transport packaging or may have enhanced features.

Nonfunctional Packaging

In case of nonfunctional packaging the closures are put in single or multiple bags and the bags then are placed into cartons or some type of bulk packaging. Other than just the containment of the closures the bags also take care of preserving their state of particulate cleanliness. Bags of this type are simple polyethylene bags that themselves of course should not shed particles or fibers.

The pharmaceutical user will unpack the closures from the bags and, in case of aseptic filling, transfer them to containers that are compatible with their own sterilization process. These may be containers that are placed in an autoclave. Alternatively the pharmaceutical user may decide to rewash the closures.

Functional Packaging

In case of functional packaging the bags that contain the closures have an additional function at the time of sterilization of the closures. In case of steam sterilization one speaks of packaging "ready for sterilization," in case of irradiation sterilization the term RtU packaging is used.

Packaging Ready for Sterilization

The function of "RfS" bags is that the same bags are used to contain the closures during transport and during steam sterilization. In this case the pharmaceutical user will unpack the RfS bags with the closures from their protective wrapping and transfer them directly into his autoclave for steam sterilization. No rewashing of closures is undertaken.

RfS bags thus must have the following properties:

- They must resist autoclave conditions. RfS bags that currently are in the market resist to temperatures up to 125°C. They are compatible with steam sterilization at 121°C, but not at 134°C. Above 125°C they start to weaken and eventually melt.
- They must be permeable to gases. They must allow air to be evacuated during the vacuum phase at the beginning of the steam sterilization process. Then they must permit steam to enter into the bag to have its sterilizing action. During the drying phase at the end of the autoclave cycle they must allow water vapor to be evaporated.

Figure 11 Picture of a ready for sterilization bag. The bag on the bottom has its Tyvek side up; the bag on top has its non Tyvek side up.

- They must be impermeable to microbiological contamination. At the end of the sterilization cycle the closures in the bags are sterile. The bag must be able to guarantee that no microbiological recontamination takes place.

The market offers many types of RfS bags. The ones that are used for steam sterilization of elastomeric closures are composed of two layers of polyethylene in different physical form that are welded together. The welding must be very solid since the weight of the closures in the bag is considerable. One layer of the RfS bag consists of a nonwoven form of polyethylene that is known in the market as "Tyvek." Tyvek has the unique property of being permeable to gases, but not to microbial contamination. The second layer of the bag consists of a regular form of polyethylene that has high enough temperature resistance. This layer is not permeable to gases, nor to microbiological contamination.

It is clear that RfS bags need to have a defined level of particulate cleanliness (Fig. 11).

Packaging Ready to Use

RtU bags are suitable for γ irradiation of elastomeric closures. The closure manufacturer will after washing and drying pack the closures in the RtU bags and provide these bags with protective overwrapping in the form of one or more regular polyethylene bags. From there the closures are transported to an irradiator contractor who performs the γ sterilization of the closures. The pharmaceutical user who is the last in the chain will take off the protective wrapping from the RtU bags and transfer the closures directly to the filling lines in their sterile area. No rewashing nor sterilization of closures is undertaken. As such, RtU bags must be impermeable to microbial contamination.

RtU bags may be made of different types of polymers. Polyethylene can be sufficient since γ irradiation does not have a destructive effect on it. Other types of bags are however possible.

Rapid Transfer Port Packaging

A special case of functional packaging that is gaining more and more attention is rapid transfer port (RTP) packaging. Such packaging is designed to be easily connectable to dedicated ports on isolators or "restricted access barrier systems" (RABS). RTP packaging for elastomeric closures exists in both irradiation sterilization and in steam sterilization compatible forms. RTP bags will always have a "collar" integrated into them. This collar is the mobile part of a two-component system of which the port on the isolator or RABS is the fixed part. When the collar is docked onto the port a system is created that allows aseptic transfer of the sterilized components contained in the RTP packaging into the isolator or RABS (Fig. 12).

Packaging Validation

Validation of the packaging of elastomeric closures in particular is of relevance for functional packaging. At some point in their life cycle such packaging will contain sterile products. The validation of functional packaging comes down to yielding evidence that this packaging is

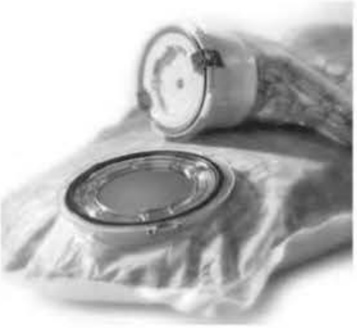

Figure 12 Picture of two different rapid transfer port bags. The collars are intended to be docked onto a restricted access barrier system or isolator port.

"tight and strong," both before and after sterilization. Microbial tightness of the packaging is important because ingress of microbiological contamination must be avoided before sterilization and of course recontamination after sterilization must be avoided at all times. Apart from choosing the correct materials for construction of the bag, assuring bag tightness can be obtained by developing suitable sealing processes after packing of the closures. The heat-sealing process for the bag shall be capable of generating a seal that is tight before sterilization and that is not affected by the steam sterilization or γ irradiation process. Demonstration of tightness of the seal can be done using microbiological methods or physical methods as a dye ingress method. Equally the sealing process shall generate a seal that is sufficiently strong to resist the weight of the closures, the stress during the steam sterilization process and the handling that inevitably is associated with the bags. Demonstration of the strength of the seals can be given by measuring tear strength of the seals. In the case of γ irradiation it shall equally be demonstrated that there is no effect of time after irradiation on seal strength. Validation of RTP packaging involves demonstration of tightness and strength of yet another seal, namely that of the collar on the bag material. Other points in validation of functional packaging may relate to particulate cleanliness of the bags and in case of γ irradiation to yielding data about discoloration of the bags after irradiation.

QUALITY CONTROL AND QUALITY ASSURANCE IN ELASTOMERIC CLOSURE MANUFACTURING

In-Process Control

Many controls can and will be executed during the manufacturing of elastomeric closures. They range for instance from checking weight on preforms to in-process monitoring of component height, to a visual check of the trimming edge of freshly trimmed stoppers. It is up to the closure manufacturer to determine which particular controls are deemed to be significant and should consequently be performed and documented.

The present paragraph does not intend to discuss further the aforementioned types of controls. Instead a further discussion will be made on controls that generally are formally carried out and documented by qualified people from a quality department.

Included in the category of in-process-controls are tests that serve to confirm the identity of the material that is being processed. Particularly after mixing or preforming, the manufacturer wants to confirm by testing that the material displays all the intended identity characteristics. This is possible by taking samples of the mixed or preformed material and by verifying physical and chemical properties on appropriate test plates made from it.

Physical properties may include a selection or the totality of the following tests:

- Specific gravity
- Ash percentage
- Hardness
- Aspect (assessment of color and homogeneity)
- Rheometry

It is to be noted that the aforementioned tests include only properties that can be affected by the weighing, mixing and preforming operations and do not relate to pure material properties such as gas permeability.

Chemical properties may include a selection or the totality of tests performed according to a standardized method such as USP <381>, Pharm. Eur. 3.2.9 or ISO 8871-1.

None of the aforementioned determinations is capable of confirming on its own the identity of the rubber material. However, every determination leaves its fingerprint and by combining the results of all tests the identity of a rubber compound can undisputedly be confirmed.

In addition to confirming the identity of the material, by carrying out these tests, data are generated that may be used for compiling the Certificate of Analysis or Certificate of Conformity of the product batches that result from the material.

Finished Product Inspection

The term finished product inspection describes the activities that are carried out on closures at the end of the manufacturing process. The tests at this stage comprise a selection, or if applicable the totality, of the following tests:

- Visual inspection of a sample of the inspected batch for the presence of *cosmetic defects*. Included in the category of cosmetic defects are only those defects that constitute a cosmetic failure and that will not influence the functional performance of the part. Cosmetic defects may be further subdivided into critical, major or minor, usually on the basis of their size. At any rate, if such subdivision is made an appropriate definition of the different classes needs to be made.
- Visual inspection of a sample of the inspected batch for the presence of *functional defects*. Functional defects are those defects that with a certain likelihood could lead to inadequate functional performance of the part. They may also be subdivided into critical, major and minor. Again, definitions of "critical," "major," and "minor" need to be established, whereby it is logical that critical defects must not be present in the sample.
- Check on a sample of the inspected batch for *dimensional compliance* with the product drawing. A distinction can be made here between product dimensions that are affected by the manufacturing operations of the part or those that are not affected by the manufacturing process. A typical example of the former class is the total height of a part; a typical example of the latter class is the depth of a product cavity that is determined by the mold dimensions only, and not by the molding operation. Finished product inspection will at least check a dimension that is affected by the manufacturing process, typically total height or flange thickness.
- Check on *functional performance*. In the case of a stopper, such tests can consist of determining coring, self-sealing, and penetration characteristics. Product specific testing may also be introduced under this heading, such as the determination of the holding force of needle shields on prefilled syringe barrels.
- Check on *surface siliconization* (for siliconized parts). This check may be carried out using a chemical analytical technique or may just consist of an assessment based on comparison with parts of known siliconization degree.
- Check on *particulate cleanliness*. Such a check includes the determination of visible and/or subvisible particulate cleanliness on a sample of the batch.
- Check on *microbiological cleanliness*. This check entails the determination of the bioburden and/or endotoxin load on a sample of the batch.
- *Chemical testing*. The manufacturer may decide to document chemical cleanliness of the material on finished product and not in-process. For coated parts, incorporating chemical cleanliness testing as part of finished product inspection testing is most logical.

Finished product inspection levels are usually taken from standards such as ISO 2859-1, "Sampling procedures for inspection by attributes Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection," for which the still much cited Military

Standard MIL-STD-105E has served as a basis. Both standards use the concept of "AQL" or "acceptable quality limit." The basis for the acceptance of a product batch is the occurrence of an acceptable number of defects in a statistical sample of the batch, whereas the rejection of a product batch is based on the occurrence of a number of defects that exceeds the acceptable limit. Sampling schemes, sample sizes, number of accepted defects, etc., are regulated by the standard.

Every user of elastomeric closures of course is permitted to make his own listing of defects to which he attributes acceptability or nonacceptability. A potentially useful, although not in all aspects up-to-date, reference that may be helpful in this respect is the "Defect Evaluation List for Rubber Parts," edited by Editio Cantor in Germany. This list has been compiled by a consortium of major German pharmaceutical companies that are active in parenterals.

Quality Systems

It is typical for elastomeric closure manufacturers to maintain a Quality System as per ISO 9001, "Quality management systems Requirements." This system will usually cover their manufacturing, testing, sales and R&D activities. Apart from the normative aspects of ISO 9001, the Quality System will contain elements of current Good Manufacturing Practice (cGMP) that are typical for the pharmaceutical industry and that many times go beyond the scope of ISO 9001. Until recently every manufacturer at its own discretion included those elements that he thought were pertinent. An emphasis thereby typically was on traceability and on disposition status (released/rejected/quarantined) of raw materials, in-process materials and finished materials. A more comprehensive guideline in this respect has been offered by ISO 15378, "Primary packaging materials for medicinal products Particular requirements for the application of ISO 9001:2000, with reference to Good Manufacturing Practice (GMP)." Certification against this relatively new standard is finding acceptance with elastomeric closure manufacturers.

STANDARDS FOR ELASTOMERIC CLOSURES FOR PARENTERALS

There are many standards that relate to elastomeric closures for parenteral use. In some cases this relation is very explicit as in pharmacopeia and ISO standards, however in some cases as FDA Guidances the relation can be less explicit. In this paragraph only a discussion of pharmacopeial sections related to elastomeric closure testing is given, as well as a listing of the most relevant ISO standards.

Pharmacopeia

There are three major pharmacopeia that impose requirements on elastomeric closures for parenterals: USP, Pharm. Eur., and Pharm. Jap. The relevant sections are USP <381>, Pharm. Eur. 3.2.9 and Pharm. Jap. 7.03. The types of tests that are contained are as listed in the table below.

	Chemical (extractables)	Functional	Biological
USP <381>	Yes As from May 1, 2009 on aqueous extract only and large degree of alignment with Pharm. Eur.	Yes As from May 1, 2009 on fully harmonized with Pharm. Eur.	Yes, through reference to USP <87> and USP <88>
Pharm. Eur. 3.2.9	Yes	Yes	No
Japanese Pharmacopeia **7.03**	Yes No harmonization with USP and Pharm. Eur.	No	Yes (hemolysis and pyrogens)

Abbreviations: USP, U.S. Pharmacopeia; Pharm. Eur., European Pharmacopeia.

ISO Standards

- ISO 247: Rubber Determination of ash
- ISO 2230: Rubber products Guidelines for storage
- ISO 2859-1: Sampling procedures for inspection by attributes Part 1: Sampling schemes indexed by acceptance quality limit (AQL) for lot-by-lot inspection

- ISO 7619-1: Rubber, vulcanized or thermoplastic Determination of indentation hardness Part 1: Durometer method (Shore hardness)
- ISO 8362-2: Injection containers for injectables and accessories Part 2: Closures for injection vials
- ISO 8362-5: Injection containers for injectables and accessories Part 5: Freeze drying closures for injection vials
- ISO 8536-2: Infusion equipment for medical use Part 2: Closures for infusion bottles
- ISO 8536-6: Infusion equipment for medical use Part 6: Freeze drying closures for infusion bottles
- ISO 8871-1: Elastomeric parts for parenterals and for devices for pharmaceutical use Part 1: Extractables in aqueous autoclavates
- ISO 8871-2: Elastomeric parts for parenterals and for devices for pharmaceutical use Part 2: Identification and characterization
- ISO 8871-3: Elastomeric parts for parenterals and for devices for pharmaceutical use Part 3: Determination of released-particle count
- ISO 8871-4: Elastomeric parts for parenterals and for devices for pharmaceutical use Part 4: Biological requirements and test methods
- ISO 8871-5: Elastomeric parts for parenterals and for devices for pharmaceutical use Part 5: Functional requirements and testing
- ISO 9001: Quality management systems Requirements
- ISO 11040-2: Prefilled syringes Part 2: Plungers and discs for dental local anaesthetic cartridges
- ISO 11040-5: Prefilled syringes Part 5: Plungers for injectables
- ISO 11137: Sterilization of health care products Radiation (3 parts)
- ISO 11608: Pen-injectors for medical use (3 parts)
- ISO 13926-2: Pen systems Part 2: Plungers and discs for pen-injectors for medical use
- ISO 14644-1: Cleanrooms and associated controlled environments Part 1: Classification of air cleanliness
- ISO 15378: Primary packaging materials for medicinal products Particular requirements for the application of ISO 9001:2000, with reference to Good Manufacturing Practice (GMP)
- ISO 15759: Medical infusion equipment Plastics caps with inserted elastomeric liner for containers manufactured by the blow-fill-seal (BFS) process

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Thanks, Lisa, for reviewing this text!

14

Parenteral product container closure integrity testing

Dana Morton Guazzo

INTRODUCTION

The definition of container closure integrity is simply, the ability of a package to adequately contain its contents by preventing content loss or contamination. This basic description is clear and straightforward. But the concept of container closure integrity is surprisingly complicated given the variety and complexity of parenteral product dosage forms and their packaging.

The demands placed on parenteral product packaging often exceed the requirements of other dosage form containers. Clearly, all pharmaceutical product package systems must prevent content leakage or spillage. But for some parenteral product packages, product loss includes vacuum loss or escape of inert gases or solvent vapors. All pharmaceutical packages must prevent contamination from environmental dirt or debris. However, parenteral product packages must also preclude microorganism contamination. And for some parenteral products, contamination may include unwanted chemicals, even moisture, originating from the outside environment or leaching from the package components themselves.

Another complicating factor of parenteral container closure integrity is the multiplicity of parenteral package designs. For instance, many products are contained in vial package systems. A typical vial package is comprised of a glass or plastic vial or bottle stoppered with a viscoelastic closure compressed against the vial mouth and held in place via a crimped aluminum cap. Prefilled syringes and cartridges, made of either glass or plastic, are becoming increasingly popular. Such systems include a closure or plunger that must adequately contain and protect the contents but must still glide smoothly along the barrel wall at time of drug delivery. The delivery port for cartridges and syringes consists of either an adhesively bonded needle covered with an elastomeric shield, or a luer tip protected with an elastomeric or plastic closure. Flame-sealed glass ampoules were once very common, but are infrequently used for today's new products. On the other hand, plastic blow-fill-seal (BFS) ampoules often package nebulizer solution preparations. Ophthalmic solution products are primarily contained in plastic bottles with uniquely designed plastic caps for easy product use. The closure mechanisms of such bottle/cap systems often include screw-threaded closures and plug- or compression-fitted components. Larger volume intravenous infusion solutions are typically packaged in plastic bags with elastomeric ports for spike access, held together via heat seals and/or ultrasonic welds.

Taking one step back, many parenteral product formulations, and even active ingredients, must be aseptically stored prior to filling into the final product package system. Such bulk storage systems must meet critical package integrity criteria. To make matters even more challenging, finished product, bulk formulation and active substance package systems vary extensively in design and materials of construction.

Given the diversity of packages, products, and integrity requirements, it is no surprise that a universally acceptable container closure integrity test method is nonexistent. Even selecting one appropriate method for any given product package system can be daunting. Much discussion and research over the last three decades has focused on identifying and validating suitable parenteral product container closure integrity test methods for some of the more common packages. Microbial challenge tests continue to be used, although a growing number of approaches for leak testing packages by physicochemical methods are available. When validating a physicochemical container closure integrity method, debate continues on the need for a comparison study against a more traditional microorganism challenge test, how to perform such a comparison, and what should be the acceptance criterion.

Fortunately, consensus on how to evaluate the integrity of at least some parenteral product packages appears to be evolving. This chapter will attempt to introduce container closure integrity concepts as they relate to some of the more widely used parenteral product packages, and to share new directions in finished product parenteral package integrity verification.

PACKAGE SEAL CHARACTERIZATION AND OPTIMIZATION

Package closure is effected either by physically mating package components or by chemically bonding them together. To ensure adequate container closure integrity, package design and development should include both theoretical and practical closure characterization and optimization studies. A clear understanding of critical component dimensions, materials of construction, and design enables the establishment of appropriate component purchasing specifications and quality controls. Package integrity studies during later development stages should also incorporate packages assembled according to actual or simulated manufacturing operation conditions. Containers assembled by hand or using laboratory scale equipment may not perform comparably to those assembled on automated, high speed manufacturing lines.

Mechanically Fitted Seals

Mechanically fitted components rely on precise dimensional fit, adequate compression, and/or tortuous paths for seal integrity. Therefore, component dimensions and tolerances should ensure the worse case "loosest" fit will still preclude leakage gaps, while the worse case "tightest" fit will permit successful, damage-free package assembly. Checking component dimensional specifications and tolerances provides a theoretical analysis of worse case component fit. However, package assembly line trials performed under anticipated manufacturing conditions play an important role in package integrity validation.

The vial/elastomeric closure/aluminum seal parenteral package (vial package) is an excellent example of a mechanically sealed package. The plug dimension of an elastomeric closure for a vial package should be sufficiently narrow to allow easy insertion into the vial neck, and so minimize vial breakage or closure "pop-up." Then again, some compression is necessary if the package must maintain an inert gas or vacuum atmosphere prior to aluminum seal capping. Elastomeric closure design, formulation, lubrication and polymer coatings all influence stopper insertion and closure-plug/vial-neck seal integrity. The vial throat dimension and design (i.e., absence or presence of a locking ring or "blow-back" feature) also significantly impact stoppered vial integrity and machinability. Finally, the aluminum seal height should be long enough to allow proper seal tuck under the stoppered vial flange, but not be so long that assembled packages exhibit inadequate closure flange compression. All these factors make a purely theoretical evaluation of such a package's closure mechanisms nearly impossible. Often vial, closure, and seal components are sourced from multiple suppliers making it difficult to ensure an optimally designed fit given all possible component combinations. Some pharmaceutical firms use computer modeling software to simulate closure compression during vial-neck insertion and seal capping. Certainly, such tools are useful, but the only way to be confident of a package's leak tightness is to integrity test finished containers, representing multiple component lots assembled at manufacturing line operational limits, using appropriately sensitive test methods. Reportedly, a few firms have gone so far as use vials made to worst case dimensions, and closures lubricated to either extreme for such studies.

Another example of a mechanically sealed system is the ophthalmic dropper-tip bottle with a screw-cap closure. Typically, the dropper-tip base snaps into the bottle neck creating a valve seal fitting. The other critical seal occurs where the inner top surface of the torqued cap presses down against the dropper-tip opening. Small shifts from optimum component designs or dimensions at these critical locations can have disastrous results. Plastic resin changes may affect component viscoelasticity which ultimately can also impact package integrity. For example, the screw cap may back off and/or component polymer creep may occur over time, especially upon exposure to temperature swings, shock or vibration. To ensure package integrity, assembled container leak test methods should identify leakage from these critical sealing locations. Supplier specifications and controls should be in place to ensure that molded components are made from approved materials, and that they conform to dimensional tolerance limits and to absence of defects specifications. Ophthalmic package production line assembly trials prior to product launch can help identify unanticipated problems. For instance, marketed product-package integrity failures have resulted from incomplete insertion of the dropper tip into the bottle neck, insufficient or excessive screw-cap torque force, and gaps at the dropper-tip/torqued-cap sealing interface.

A syringe or cartridge has a mechanically fitted closure (also called a "plunger") positioned inside the syringe/cartridge barrel to prevent content leakage, yet is designed to glide smoothly with minimal resistance at time of drug delivery. The dimensions of the closure and barrel, and the closure's viscoelastic properties determine this mechanical seal's effectiveness. The amount of lubrication on the barrel wall and the closure also impacts closure performance. For this reason, studies to evaluate both syringe leakage and functionality may use components made to simulate tightest and loosest fit, lubricated and sterilized under the most challenging anticipated conditions.

Chemically Bonded Seals

Chemical bonding techniques are used for sealing various pharmaceutical packages. Heat sealing using thermal impulse or conductive heat sealers is one such technique. Examples of packages sealed in this manner include plastic bags for sterile powder storage, and barrier laminate pouches for protecting semi-permeable plastic BFS ampoules. Consistent seal strength and barrier properties rely on proper characterization and control of heat seal layer polymer composition, molecular structure, and laminate thickness. In addition, the heat sealing process critical parameters of heating, cooling, pressure and time should be controlled and monitored within optimized ranges along the entire length of the seal.

Ultrasonic welding is another well-known process used to create polymer-polymer seals for pharmaceutical packages, although other industries use this technique to bond metals to plastics or even metals to metals. Ultrasonic welding is very fast and usually produces welds relatively free of flash making it attractive in clean room settings. A welding tool transmits ultrasonic energy to the part to be bonded, causing mechanical vibration and frictional heat at the sealing interface. Rapid melting and bonding occurs at the connecting surfaces statically pressed together. Effective ultrasonic welding requires that the bonded polymer materials exhibit nearly equivalent melting points. Amorphous thermoplastics weld more efficiently than semicrystalline materials, harder materials with high modulus are also easier to weld. Thus, consistent welding requires proper characterization and control of polymer layers' thickness, composition and molecular structure. Optimization and control of ultrasonic frequency, oscillation amplitude, power level and pressures are vital, as well as the tool design used to direct energy between the welded parts.

Adhesives can also accomplish a chemical bond between package surfaces. For example, UV and visible light curing adhesives effect the bond between stainless steel needles and the tips of glass or plastic syringe barrels. Semi-rigid plastic trays used for many medical devices or drug-device combination kits often incorporate porous barrier lidding materials, such as Tyvek® or low-linting papers, bonded to the tray with a heat-activated adhesive. Well-sealed bonds depend on the adhesive's chemical composition and quality, the adhesive application process, and the curing process, as well as the nature and quality of the bonding surfaces.

Contiguous containers, such as flame-sealed glass ampoules, represent another chemical bonding process. Glass ampoules filled with product are sealed by one of two methods. In the first case, the ampoule's stem is flame-heated at the intended point of closure. As the distal tip is pulled away the stem narrows and closes. The second glass ampoule sealing process involves heating the ampoule's open end until the glass softens and closes under gravity. Ampoule seal integrity and quality is a function of several factors, including glass formulation, ampoule wall thickness, line speed, ampoule rotation speed, ampoule tip "draw" speed (if applicable), and flame heat. Typical glass ampoule defects include cracks, as well as pinholes, channels, and weak, thin-wall areas usually located at the sealed tip.

Plastic BFS ampoules, another type of contiguous container, are created, filled and sealed in one continuous, aseptic manufacturing process. Dosage forms packaged in BFS ampoules include unit-dose sterile solution products, such as nebulizer solutions and intravenous line flushing solutions. Integrity of these packages is a function of the plastic formulation and the forming/sealing parameters of time, pressure and temperature. Defects that can result in package leakage include pinholes, thin-wall areas, and burrs or other contaminants trapped in the plastic wall.

LEAKAGE THEORY

Leakage occurs when a discontinuity or gap exists in the wall of a package that allows the passage of gas under the action of a pressure or concentration differential existing across the package wall. Leakage differs from permeation, which is the flow of matter through the barrier itself. Both leakage and permeation play vital roles in the study of parenteral product package integrity.

Permeation

Permeation is passage of a fluid into, through and out of a solid barrier having no holes large enough to permit more than a small fraction of the molecules to pass through any one hole. The process always involves diffusion through a solid, and may involve other phenomena such as adsorption, migration, solution, dissociation, and desorption. Permeation rate is a function of the permeant's concentration, its solubility in the barrier material, as well as the molecule's physical ability to migrate through the barrier.

The general equation for permeation is given by equation (1), where Q, the mass flow rate (Pa m^3/sec m^2) is a function of the permeation rate constant (K_P), which is a product of the solubility coefficient (S), and the diffusion coefficient (D). Permeation is directly proportional to A, the area normal to permeation flow (m^2), and ΔP, the partial pressure drop across the flow path (Pa), while inversely proportional to l, the path flow length (m) (1).

$$Q = K_P A(\Delta P/l) = (SD)A(\Delta P/l) \tag{1}$$

Permeation plays a role in package integrity assurance if the package must prevent loss of critical headspace gases or vacuum, restrict loss of product solvents or other permeable ingredients, or limit migration of external gases or vapors into the package. For example, small volume plastic BFS ampoules containing nebulizer solution are generally semi-permeable containers requiring a barrier laminate pouch secondary package to prevent the product from drying out over shelf life. Packages for hygroscopic lyophilized products or aseptically filled powders must limit moisture ingress from the outside environment or even from the package components themselves. Pharmaceutical products subject to oxidative degradation must be contained in packages that limit oxygen permeation. Some lyophilized products in vial packages require a vacuum headspace to help draw diluent into the vial upon reconstitution. Therefore, atmospheric gas permeation leading to loss of vacuum can make product use difficult and may cause end-users to question product quality.

Leakage Flux

Diffusion

Leakage is defined as the movement of molecules by convection plus diffusion through one or more gaps in the package barrier wall. The driving force for gas or liquid convective flow through a leak path is the pressure differential that exists across the barrier. If no pressure differential exists, only the concentration gradient of the leaking molecule existing across the barrier drives molecular flux according to diffusional flow kinetics.

Gas diffusion follows Fick's laws of diffusion (2). Fick's first law defines diffusion assuming a plane of infinitely small thickness [eq. (2)]. The negative sign means that when $\delta C/\delta x$ is positive, flux is in the direction of decreasing x or decreasing concentration.

$$J = \quad D(\delta C/\delta x)_t \tag{2}$$

where

J	amount of diffusion	$g/m^2 \cdot sec$
D	diffusion constant	$m^2 \cdot sec$
C	diffusant concentration	g/m^3
x	barrier thickness	m
t	time	sec

Fick's second law takes into consideration a barrier of measurable thickness, where the diffusant concentration varies across the barrier thickness and changes continually over time, thus changing the rate of flux.

$$\delta C/\delta t = D(\delta^2 C/\delta x^2) \tag{3}$$

An example of diffusional flux occurs in a parenteral vial package sealed under a nitrogen blanket. In this case, the vial interior contains a higher concentration of nitrogen and a lower concentration of oxygen than exist outside. Thus, nitrogen gas will tend to diffuse out of the vial, while oxygen will tend to leak into the vial. This tendency is especially true for stoppered vials prior to aluminum seal capping. While studies may show a stoppered vial capable of preventing ingress of relatively large air-borne microorganisms, gas molecules will readily diffuse across the tiniest leak paths.

Convection

For the most part, parenteral package integrity is concerned with fully assembled container closure systems, where measurable leakage linked to either dosage form loss or microbial ingress is chiefly convective, with little or no diffusional flow. So for the remaining discussion, unless otherwise specified, the term "leakage" refers to convective flow of gases moving from higher to lower pressure sides of a package boundary, without diffusional flux or permeation components.

Different physical laws relate leakage rate to the differential pressure gradient across the leak, the range of absolute pressure involved, and the nature of the gas moving through the leak. The five main types of pneumatic gas leak flow are turbulent, laminar, molecular, transitional, and choked flow. Approximate gas flow rates for these pneumatic modes are as follows (1):

1.	Turbulent flow	$>10^{\ 3}$	Pa m^3/sec
2.	Laminar flow	$10^{\ 2}\ 10^{\ 7}$	Pa m^3/sec
3.	Molecular	$<10^{\ 6}$	Pa m^3/sec
4.	Transitional	Between molecular and laminar	
5.	Choked	When flow velocity approaches the speed of sound in the gas	

Laminar and turbulent flow are both classes of viscous flow. Because turbulent flow is rarely encountered in leaks, the term viscous flow is sometimes incorrectly used to describe laminar flow. This chapter focuses on leakage ranging from turbulent to molecular flow the leak rates of greatest concern for most nonporous parenteral packages. Laminar flow occurs when the mean free path length of the gas (λ) is significantly smaller than the leak path's cross-sectional diameter ($\lambda/d < 0.01$). The mean free path length is that at the average pressure within the leaking system. The leak rate (Q) follows Poiseuille's law for laminar flow through a cylindrical tube (1).

$$Q = [(\pi r^4)/(8nl)][P_a(P_1 \quad P_2)] \tag{4}$$

or

$$Q = [(\pi r^4)/(16nl)][(P_1^2 \quad P_2^2)] \tag{5}$$

where

Q	gas flow rate	Pa m^3/sec
r	leak path radius	m
l	leak path length	m
n	leaking gas viscosity	Pa sec
P_1	upstream pressure	Pa
P_2	downstream pressure	Pa
P_a	average leak path pressure, $\frac{(P_1+P_2)}{2}$	Pa

Molecular flow occurs when the mean free path length of the gas is greater than the cross-sectional diameter of the leak path ($\lambda/d > 1.00$). Molecular flow leak rates are defined according to Knudsen's law for molecular flow through a cylindrical tube, neglecting the end effect, as per equation (6) (2). By comparing equation (6) with equations (4) and (5), it is evident that laminar flow is a function of the leaking gas's viscosity, whereas molecular flow is a function of the gas's molecular mass.

$$Q = (3.342)(r^3/l)(RT/M)^{1/2}(P_1 \quad P_2) \tag{6}$$

where

Q	gas flow rate	Pa m^3/sec
r	leak path radius	m
l	leak path length	m
M	molecular weight of leaking gas	(kg/mol)
T	absolute temperature	Kelvin
R	gas constant, 8.315	J/(mol K)
P_1	upstream pressure	Pa
P_2	downstream pressure	Pa

Transitional flow occurs when the mean free path length is about equal to the leak's cross-sectional diameter ($\lambda/d = 0.01 \quad 1.00$). The equations for transitional flow can be quite complex. For further discussion on convective flux, refer to *The Nondestructive Testing Handbook* (1).

Practical Application

Package integrity research studies utilize the above equations and concepts in a variety of useful ways. For example, a leak path's nominal width can be calculated by measuring the gas flow rate through the leak (the leak rate), assuming either molecular or laminar gas flow behavior. University of Iowa researchers measured the helium leak rate through various capillary tubes embedded in the walls of glass vials to estimate these artificial defects' diameters (3).

In another example, package leakage through a hypothetical defect can be calculated and compared with actual package leakage, thus confirming the defect's absence or presence. For instance, consider a lyophilized product sealed under vacuum conditions in a stoppered/capped vial. The lower pressure conditions in the vial act to draw air into the package through any gaps present. By knowing the vial headspace volume and the absolute pressure in the package at time of capping, the theoretical vacuum loss over time due to a given-size leak can be modeled using convective flux equations. Actual headspace pressure readings below modeled predictions confirm the vial's integrity. Similarly, Fick's laws of diffusion can predict the rate of oxygen ingress into an inert gas flushed, stoppered vial as a function of a hypothetical leak. Both of these predictive models are explored more fully later in this chapter.

Leakage Units of Measure

Leakage rate is the amount of gas (mass or volume) which passes through a leak path under specific conditions of temperature and pressure. Therefore, leakage rate has dimensions of pressure multiplied by volume, divided by time. Table 1 lists several common leak rate units of

Table 1 Mass Flow Conversion Factors for Common Leak Rate Units

Pascal cubic meter per second	Standard cubic centimeter per second	Mol per second	Millibar liter per second	Torr liter per second
Pa m^3/sec	Std cm^3/sec Alternatively, sccs	mol/sec	mbar L/sec	torr L/sec
1	9.87 ($\simeq$10)	$4.4 \times 10^{\ 4}$	1.00×10^1	7.50

Source: From Ref. 4.

measure. The international standard SI nomenclature is pascal cubic meter per second (Pa m^3/sec). To express leak rate in mass flow units, rather than volumetric flow units, the results must be converted to standard conditions of 101 kPa (760 torr) and 0°C (32°F). When expressing leakage volumetrically, test pressure and temperature conditions are specified.

PACKAGE LEAKAGE ACCEPTANCE LIMITS

Since leakage is the rate of gas flow through a leak path, it is meaningless to say that a package has zero leakage, or is leak-free without reference to a leak rate specification. This is similar to saying that a pharmaceutical ingredient is pure or a dinner plate is clean. These expressions are only meaningful when compared with some purity or cleanliness standard. In the same way, a leak-free package simply means the package does not leak above some acceptable leakage limit. The key to setting leak rate specifications is to select meaningful limits, while avoiding unreasonable, and costly requirements. Unnecessarily small leak rates limits will result in expensive instrumentation, increased test time, and rejection of otherwise acceptable product.

Setting realistic and useful leak rate specifications for parenteral products requires characterization of the package sealing mechanisms as well as an understanding of finished product dosage form specifications and the package's performance requirements. This enables logical and practical integrity test method selection. For example, all parenteral products must be sterile; therefore, all packages must be able to prevent liquid- and/or air-borne microbial ingress. All parenteral product packages must also contain the product, preventing loss. Thus, for liquid dosage forms the packaging must also prevent liquid leakage. Studies have shown that leaks that allow liquid flow are also at risk of microbial ingress; the larger the leak, the greater the risk. Conversely, when liquid cannot pass through a leak, microbes cannot (5 7). For this reason, leak tests capable of identifying the smallest leak paths able to contain liquid or permit liquid flow may serve to verify a package's microbial integrity. This microbial ingress/ liquid leakage relationship, briefly introduced at this point, is a topic explored extensively throughout this chapter.

Some leak tests, such as helium mass spectrometry, provide test results in quantitative gas flow rate terms. Therefore, when using such methods it is important to know how gas leak rates correlate to critical package performance requirements. For example, helium trace gas leak test studies have linked gas flow rates as small as about 10^{-6} Pa m^3/sec to the smallest leaks able to permit liquid leakage plus microbial ingress (8). Leak detection texts define water-tight seals as meeting limits of about 10^{-4} Pa m^3/sec, whereas, relatively large leaks from misassembled, misshapen or damaged packages are most often above 10^{-4} Pa m^3/sec (9).

Gas headspace preservation is a practical package performance requirement linked to leakage acceptance criteria. For instance, if the product requires low oxygen container headspace content, then oxygen permeation plus air leakage must remain below a specified limit. Similarly, hydroscopic product packages must limit moisture ingress. Integrity tests that specifically monitor gas or vapor migration are reasonable options in such cases. For packages sealed under negative pressure, instruments to monitor headspace pressure are preferred.

LEAK TEST METHODS

Many leak test methods exist for testing everything from soft drink cans to vacuum pumps to heart pacemakers. Even within the relatively small world of parenteral packaging, numerous leak test methods apply (10). Rather than provide an exhaustive survey of all potentially useful leak test methods, this chapter will focus on those testing techniques having the broadest application for the most common parenteral packages, namely, vial packages, prefilled syringes, ophthalmic dropper bottles, and plastic or glass ampoules.

Microbial Challenge Methods

A microbial challenge test procedure includes filling containers with either growth-supporting media or product, followed by closed container immersion in a bacterial suspension or exposure to aerosolized bacteria or bacterial spores. Test containers are incubated at conditions that promote microbial growth, and container contents are then inspected for evidence of microbial growth. Positive challenge organism growth is indicative of package leakage.

Currently, no standard microbial challenge test method exists (10). In reality, any one of many possible microbial challenge methods may prove satisfactory as long as it is scientifically sound, given the package type and its protective function, and the product's anticipated exposure to conditions of processing, distribution, and storage. The following discussion explores factors to consider when designing a microbial challenge test.

1. Challenge mode. If a package is able to tolerate liquid immersion, then this approach is generally favored for parenteral package system testing, as it presents the greatest challenge to package seals. Aerosol challenge testing is most appropriate for packages that rely on tortuous paths, or seals not intended to prevent liquid leakage. Aerosolized challenges are frequently used in the food and medical device industries. Static testing, where packages filled with media are simply stored under normal warehouse conditions or in stability storage chambers, affords no definitive bacterial challenge and no significant pressure differential to the seals. If such long term storage of media-filled units is part of an integrity verification program, then some known bacterial challenge to the packages at the end of the storage period is appropriate.
2. Challenge parameters. Liquid immersion challenge tests preferably include vacuum/pressure cycling simulating pressure variations anticipated during product life processing, distribution and storage. These cycles will enhance flow of packaged media into any leak paths present, thus encouraging potential microbial ingress. For this reason, package position during the challenge test should ensure packaged media contact with seal areas. An aerosol challenge test chamber size and design should guarantee uniform distribution of viable aerosolized bacteria or spores around the test packages, considering factors such as chamber temperature and humidity, as well as airflow patterns and speed.
3. Challenge microorganism. Liquid challenge organism size, mobility and viability in the packaged media are important factors for consideration. Bacteria concentration in the challenge media at the initial time point should ensure a high concentration of viable organisms at the test's conclusion (e.g., $\geq 10^5$ CFUs/mL at end of test). Bacteria used in published immersion challenge studies include, but are not limited to *Escherichia coli*, *Serratia marcescens*, *Clostiridium sporogenes*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Brevundimonas diminuta*. When performing aerosol challenge tests, aerosolized microorganism concentration and uniformity are important factors, as well as viability in the packaged media. Reportedly, aerosol challenge testing commonly uses *Bacillus atrophaeus* spores and *Pseudomonas fragi* microbes.
4. Growth promotion media. All challenge tests require test containers filled either with growth-promoting media or product that supports microbial growth. The product formulation itself or a product placebo is preferred as it most closely simulates the product package system. However, this may not be practical if the intention is to validate a variety of products in similar packaging. Verification of the media's growth promotion capability at the completion of the package integrity test is important, especially if the test sample holding time is lengthy.
5. Test package preparation. Two approaches are possible for preparing sterile packages for testing. Either previously sterilized package components are aseptically filled with the growth-promoting vehicle, or media-filled packages are terminally sterilized. If feasible, the sterilization procedures and package assembly processes chosen should mirror those used for the actual product. Otherwise, the test package and seal may differ in some respect from the marketed product package system. For example, vial package capped closures exhibit a certain amount of sealing force on the vial land seal surface. This residual seal force will noticeably decay upon terminal steam sterilization, thus potentially changing the seal quality (11,12). Similarly, plastic bag test samples exposed to gamma irradiation post heat sealing may not represent product bags normally sealed using ethylene oxide sterilized materials.
6. Test package quantity. There is no guarantee of microbial ingress even in the presence of relatively large defects. Microbial ingress is a notoriously probabilistic

phenomenon. For this reason, a valid test requires a relatively large population of test samples and positive controls.

7. Positive and negative controls. All leak test validation protocols, including microbial challenge tests, require positive control or known-leaking packaging in the test package population to demonstrate the test's leak detection ability. Negative controls, or so-called good packages, are also important to establish a baseline of intact package performance. Additional information on positive controls is included under a separate heading.

Microbial challenge tests have been used to verify container closure integrity for decades. However, there are problems with solely relying on this approach. First, microbial challenges, especially immersion tests, do not simulate real life, product bio-exposure conditions. Simply put, package seals are not typically soaked in media highly concentrated with microbes, while differential pressures promote liquid and microbial entrance. Yet, even under these extreme challenge conditions, the highly probabilistic nature of any microbial challenge test makes results difficult to interpret. Leak paths several fold wider than a microorganism will not guarantee microbial ingress, as numerous studies have shown (5,7,8,13). On the other hand, the rare occurrence of microbial grow-through across a package's fitted seam during an exceptionally severe biochallenge may negate the use of an otherwise acceptable container closure system, even though such a challenge does not realistically portray naturally occurring phenomena.

Conversely, inappropriately designed microbial challenge tests can easily make bad packages look good. Short exposure times; minimal or no differential pressure application; small test sample populations; and positive control packages with very large leaks all help samples with questionable seals pass a microbial challenge test, thereby falsely implying package integrity. In some cases, reliance on such tests has kept leery companies from adopting more reliable, physicochemical leak test methods, despite known product package integrity problems.

Suitably designed and executed microbial challenge tests, if used, are of greatest value during package development and early clinical research programs. Microbial challenge tests are one of the few appropriate tests for integrity verification of porous barrier materials and tortuous path closure systems. However, reliance on microbial challenge tests for most package types throughout a product's life cycle has disadvantages. Results are prone to error and the test itself consumes resources of time, space, equipment, and staff, making it much more expensive than cost of materials implies. Microbial challenge tests are not practical, for instance, for routine production lot integrity testing, for forensic investigations of recalled product, or when studying package component and assembly process variables. In addition, unless the product formulation supports microbial growth, the test cannot definitively validate the integrity of the actual product package system. Nevertheless, because parenteral packages must prevent sterility loss, microbial challenge tests will likely remain part of the package leak testing arsenal for some time to come.

Dye and Liquid Tracer Methods

A liquid tracer leak test consists of immersing test packages in a solution of either dye or other chemical tracer, then allowing time for liquid to migrate through any leaks present while pressure and/or vacuum are applied. After the liquid challenge, test packages' contents are checked for liquid leakage as evidenced by visual inspection or other appropriate analytical method. Liquid leak tests are relatively inexpensive, simple to perform and conceptually easy to understand. However, the test is destructive to the package, and results may vary considerably on the basis of several factors.

Test method parameters that promote greater liquid tracer test sensitivity include longer immersion times, increased pressure and vacuum conditions, smaller volumes inside the test package, and lower surface tension challenge liquids. On the other hand, debris in the challenge liquid may clog small leaks, and airlocks in leak paths may prevent liquid ingress. Restraining package part movement (e.g., partially filled syringes), or package expansion (e.g., flexible pouches) during vacuum exposure helps keep package internal pressure constant, thus ensuring consistent leakage driving forces.

The compatibility of the dye or tracer element with the package and its contents should be verified. Dyes may quickly fade or adsorb onto package surfaces shortly after leak testing; therefore, time gaps between testing and inspection or analysis should be limited and specified. Analytical methods for dye or tracer detection require appropriate validation. For the most reliable visual inspection results, qualified inspectors following defined inspection procedures in well-lit, controlled inspection environments are called for. Inspection procedures should dictate lighting intensity and color, inspection angle, background color(s), background luster, inspection pacing, and any comparator negative control package(s) used. Inspector qualification protocols should entail accurate segregation of packages containing trace amounts of dye from negative controls in a randomly mixed, blinded test sample population. A multisite study lead by H. Wolf demonstrated how differences in inspector capabilities and inspection environments play a significant role in interpreting dye ingress test results (14).

Numerous published leak test studies incorporate dye or liquid tracer test methods, some of which are described in section "Test Method Validation" (5,6,13). U.S. compendia (15), EU compendia (16), and ISO international standards (17) all specify methylene blue dye ingress tests for demonstrating punctured closure reseal properties. But before using such closure reseal methods for whole-package integrity testing, test parameters should be optimized and the methods validated using known positive and negative control packages. The importance of this was demonstrated in the previously cited study by Wolf et al., in which 1-mL water-filled syringes with laser-drilled defects in the barrel wall ranging in nominal diameter from 5 to 15 μm were leak tested according to the closure resealability dye ingress tests described in the U.S. and EU compendia and in ISO standards. None of these standard test methods permitted accurate identification of all defective syringes (14).

Vacuum Decay Leak Test Method

A vacuum decay leak test is a whole-package, nondestructive leak test method. Vacuum decay methods relate pressure rise, or vacuum loss, in an evacuated test chamber containing the test package to package leakage. A typical test cycle consists of placing the subject container in a test chamber, then closing the chamber and evacuating it to a predetermined vacuum level. Upon reaching this target vacuum within an allotted time segment, the test system is isolated from the vacuum source, and a short time for system equalization elapses. A defined test time segment follows for monitoring any subsequent pressure rise (vacuum decay) inside the test chamber. Rise in pressure above baseline, or background noise level, signifies package headspace gas leakage, and/or vaporization of product liquid plugging leak path(s). Total test cycle time is normally less than 30 seconds, but may vary with the test system, the product package tested, and the desired sensitivity level.

A package "fails" or "leaks" if any one of several events occurs during the vacuum decay leak test cycle. Failure modes include (*i*) failure to achieve initial target vacuum, indicative of largest leaks, (*ii*) rise in pressure above a defined reference pressure at any time throughout the test cycle, indicative of medium size leaks, or (*iii*) rise in pressure above a defined differential pressure value during the final test time segment, indicative of smallest leaks. Figure 1 illustrates these various failure modes.

The combination of test equipment, package test chamber, and testing cycle is unique to each product package system, and is identified on the basis of the package's contents (liquid or solid, with significant or little gas headspace), and the nature of the package (flexible or rigid, porous or nonporous).

Uniquely designed test chambers snugly enclose the test package, minimizing test chamber deadspace for maximum test sensitivity. Added features may be required to limit package movement or expansion during the test. For example, prefilled syringes require special fixtures to restrict plunger movement. Test chambers for flexible packages, such as bags or pouches, include flexible surfaces that conform to the package and prevent expansion that may stress package seals. Test chambers designed to test trays with porous barrier lidding have a single flexible bladder that masks gas flow through the porous barrier, allowing detection of leaks located around the seal perimeter or through the nonporous tray (19).

Test method reference parameters maximize test method sensitivity for each product package. These parameters include: Time to reach initial target vacuum, equalizing time,

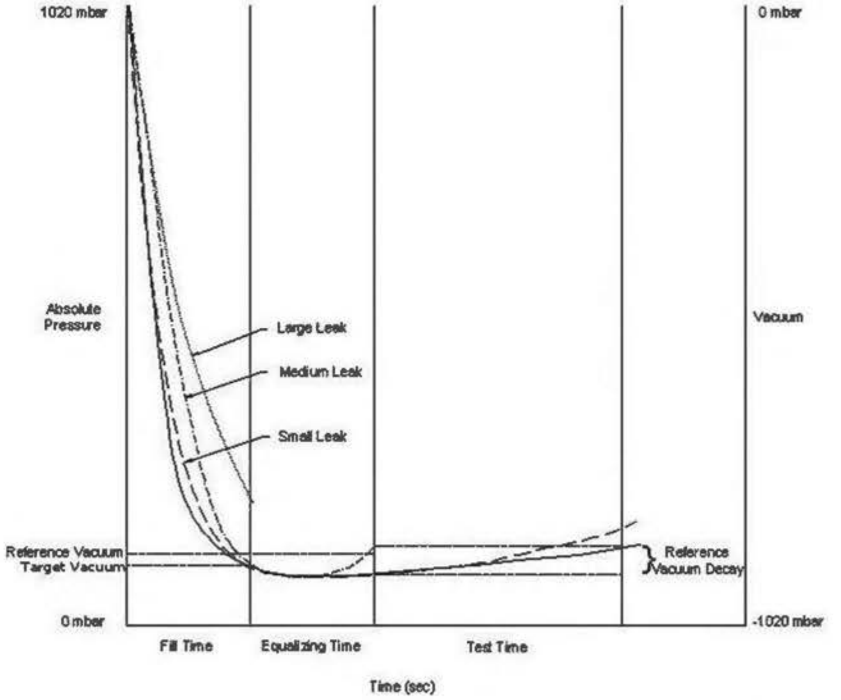


Figure 1 Pressure readings as a function of time during a vacuum decay leak test method for packages with and without leaks, according to ASTM F2338 09 *Standard Test Method for Nondestructive Detection of Leaks in Packages by Vacuum Decay Method*. *Source*: From Ref. 18.

vacuum loss test time, target vacuum level, and pressure loss limits. For instance, leaks plugged by liquid require target vacuum below the liquid's vaporization pressure, so that vaporized liquid yields a measurable rise in pressure. On the other hand, gas leaks are detectable at less severe vacuum settings. Pressure loss limits close to baseline make the test more sensitive, but run the risk of false positive test results. Generally, longer total test cycles improve test sensitivity, especially for gas leaks.

Vacuum decay leak tester designs vary among instrument manufacturers. While most models rely on a single 1000-torr gauge transducer, some instruments use a dual transducer system with either a 1000-torr gauge or absolute transducer coupled with a more sensitive, higher resolution 10-torr gauge transducer. One manufacturer that relies on the single gauge transducer approach also incorporates special software that continually readjusts the no-leak baseline to account for atmospheric pressure changes and no-leak noise variations that can affect test sensitivity. Another manufacturer is able to eliminate atmospheric pressure variation concerns and the need for calculated baseline adjustments by utilizing an absolute pressure transducer as part of their dual transducer test system (19). Automated multistation linear or rotary-style equipment enables 100% on-line testing; semi-automated or manually operated test systems with either single- or multiple-package test stations are useful for testing one or several packages simultaneously. In general, longer tests possible with off-line testers enable smaller leak detection. Thus any given vacuum decay leak test method is not only specific to the product package system, but also to the leak test instrument and its manufacturer.

Test method development and instrument functionality checks often utilize a calibrated airflow meter for artificially introducing leaks into the test chamber containing a negative, no-leak control package. Airflow meters certified by the National Institutes of Standards and Technology (NIST) or other recognized certification bodies are recommended for such purposes. The smallest rate of airflow that triggers a significantly greater rise in pressure above background noise level is the limit of detection for the leak test. However, use of calibrated airflow standards alone is not sufficient for complete test method development and validation.

For instance, consider a grossly leaking package with very small gas headspace volume. If the time allotted for reaching initial target vacuum is too long, the headspace will be rapidly lost, preventing leak detection during the pressure rise test phase. Whereas, the same test performed using a flowmeter with unlimited gas supply will still yield test phase pressure rise despite the longest chamber evacuation times. In another example, consider a plastic bottle with a pinhole-size leak in the induction seal, beneath the torqued screw-thread cap. A proper test cycle may require additional time to draw out trapped air in the cap's threads, before leakage from the induction seal hole can be observed. This phenomenon would likely be missed if test method development only used a flowmeter for leakage simulation. Further, consider the fact that leaks simulated using a calibrated flowmeter only represent gaseous leakage and not leakage from liquid-plugged leak paths. Generally, liquids clogging leaks quickly volatilize once test pressure falls below the liquid's vaporization pressure. At this point, solvent volatilization causes a rapid rise in test system pressure, which quickly stops or perhaps fluctuates once saturation partial pressure is reached. This difference in leak behavior often requires different testing parameters when checking for gas versus liquid leaks, or some combination of both.

Negative controls used for vacuum decay test method development and validation may consist of actual no-leak packages, or they may be solid material, package-shaped models. However, at some point, tests using larger populations of actual, filled, no-leak packages will ensure the baseline represents all possible package-to-package variations. Actual leaking packages filled with placebo or product are also very useful to verify the test method's ability to find various types of leaks located at various seal locations. Prior to testing actual product packages, cleaning procedures should be in place in anticipation of test equipment contamination from leaking containers.

Two vacuum decay leak test research studies reported in the literature used Wilco AG leak test systems. For both studies test samples consisted of glass vials with micropipettes affixed into the glass vials to simulate leaks. Test package leakage was quantified using helium mass spectrometry, a leak test method previously compared with liquid-borne microbial challenge tests. In the first study, air-filled vials were vacuum decay leak tested (20). The second study evaluated vials filled with various solvents that plugged the leak paths using a so-called LFC pressure rise or vacuum decay approach. This concept required the test pressure to be substantially lower than the vapor pressure of the packaged liquid (21). LFC method test results indicated potentially greater sensitivity when testing liquid-filled vials.

ASTM F2338-09 *Standard Test Method for Nondestructive Detection of Leaks in Packages by Vacuum Decay Method* (22) is a recognized consensus standard by the U.S. Food and Drug Administration (FDA), Center for Devices and Radiological Health (CDRH), effective from March 31, 2006 (22). According to the FDA Consensus Standard Recognition Notice, devices that are affected include any devices that are sterilized and packaged. Packages that may be nondestructively tested by this method include: Rigid and semi-rigid nonlidded trays; trays or cups sealed with porous barrier lidding materials; rigid, nonporous packages; and flexible, nonporous packages.

The ASTM method includes precision and bias (P&B) statements for various types of packages based on round robin studies performed at multiple test sites with multiple instruments. P&B studies have looked at porous lidded plastic trays, unlidded trays and induction-sealed plastic bottles with screw caps. The most recent P&B studies used glass prefilled syringes. Test packages included empty syringes, simulating gas leaks; and water-filled syringes, simulating leaks plugged with liquid (liquid leaks). Laser-drilled holes in the syringes' glass barrel walls ranging from 5 to 15 μm in nominal diameter served as positive control leaks. The leak testers used incorporated an absolute 1000-torr transducer coupled with a 10-torr differential transducer, manufactured by Packaging Technologies & Inspection, LLC of Tuckahoe (New York, U.S.). Two different test cycles were explored; one with a target vacuum of 250 mbar absolute for testing gas leaks only, and another with a target vacuum of about 1 mbar absolute for testing both gas and liquid leaks. Results showed the leak tests reliably identified holes as small as 5 μm in both air-filled and water-filled syringes (23).

In summary, vacuum decay is a rapid, noninvasive and nondestructive leak test method. Depending on the test system, holes as small as 5 µm in a variety of nonporous, rigid packages are reliably detected. Vacuum decay is a practical tool for optimizing package-sealing parameters and for comparatively evaluating various packages and materials. Test methods are suitable as a stability program integrity test or as an in-process check of clinical or commercial manufacturing lots. Larger scale, on-line equipment may be used for 100% production lot testing, although leak test sensitivity is considerably less than for the most sensitive off-line instruments.

Electrical Conductivity Leak Test

Electrical conductivity testing relies on the application of a high frequency electrical current near the test package. Any liquid of greater conductivity than the package material present in or near a leak path located near the detector will trigger a spike in measured conductivity (Fig. 2). Conductivity spikes occur even if leak paths are clogged with dried product an advantage not shared with other test methods that require an open leak path. This approach for testing liquid-filled packages has the added benefits of being extremely rapid, nondestructive and clean.

Electrical conductivity testing is appropriate for a wide variety of container closure systems, including plastic or glass ampoules, vial packages, prefilled syringes, and liquid-filled pouches. Electrical conductivity is not appropriate for testing flammable liquid products. In addition, only leak paths near detectors are identifiable; therefore, either package surfaces are checked using multiple detectors, or only the areas of greatest risk for leakage are monitored. Package rotation during testing may be required to capture defects around a package's circumference. Test method validation for a given product package requires demonstration of the test's ability to detect leaks at all likely package locations.

The electrical conductivity test, also known as high-voltage leak detection (HVLD), is widely employed for 100% on-line testing of plastic BFS ampoules and glass ampoules. Möll and colleagues described test method development and validation of an electrical conductivity test used for gel-filled low density polyethylene ampoules (24). Positive controls consisted of ampoules with laser-drilled holes positioned at the most likely zones for leaks to occur: the sealing zone at the ampoule bottom, and the top tear-off area. The voltage setting and the sensitivity or "gain" setting were the two parameters optimized to establish a window of operation that finds all defective ampoules and rejects few, if any, good ampoules. Replicate testing of a randomized population of negative and positive control test samples took place over three days. Each day of operation the HVLD test successfully "failed" all 210 positive control ampoules (150: 5 10 µm; 60: 10 20 µm), and "passed" 3830 negative controls. A dye ingress test confirmed the presence of defects in two of three so-called negative controls consistently rejected by HVLD. Therefore, the electrical conductivity test correctly identified all defective units and falsely rejected only one negative control sample.

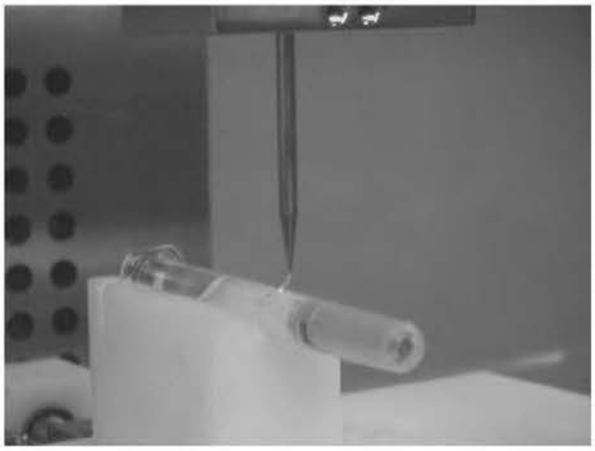


Figure 2 A glass prefilled syringe containing an aqueous liquid being tested using Nikka Densok's electrical conductivity method. Positive electrical current occurred near a laser drilled hole in the glass barrel wall. *Source*: Courtesy of Nikka Densok, Inc., Lakewood, Colorado, U.S.

Frequency Modulation Spectroscopy

Frequency-modulated spectroscopy (FMS) is a rapid, nondestructive analytical method suitable for monitoring oxygen and water vapor concentrations as well as evacuated pressure levels in the headspace of sterile product containers. Frequency modulation spectroscopy was developed in academic and industrial laboratories in the 1980s and 1990s. Over the last 10 years, the technology has found commercial application in the pharmaceutical industry for leak detection (25), moisture monitoring (26) and oxygen monitoring (27). Systems for rapid nondestructive headspace analysis were first introduced to the pharmaceutical industry in 2000 (28), and are now routinely used in product development, process development and commercial manufacturing.

The key to these test systems are diode laser devices fabricated to emit wavelengths in the red and near-infrared regions of the electromagnetic spectrum where molecules such as oxygen and moisture absorb light. Containers made of glass (amber or colorless) as well as translucent plastics allow the transmission of near IR diode laser light and are compatible with FMS test methods.

The underlying principle of laser absorption spectroscopy is that the amount of light absorbed by a molecule at a particular wavelength is proportional to the gas concentration and the gas pressure. Therefore, FMS technology works by tuning the wavelength of light to match the internal absorption wavelength of a molecule and recovering a signal where the amplitude is linearly proportional to gas density (e.g., headspace oxygen and moisture) and the signal width is linearly proportional to gas pressure (e.g., vacuum level in the headspace of a sealed vial). Figure 3 presents a simple schematic of the FMS technique. Laser passes through the gas headspace region of a sealed package; light is absorbed as a function of gas concentration and pressure; the absorption information is processed using phase sensitive detection techniques; a mixer demodulates the radio frequency signal; the output voltage, proportional to the absorption lineshape, is digitally converted and further analyzed by a microprocessor, yielding final test results.

Examples of demodulated absorption signals for headspace oxygen, moisture and total pressure are shown in Figures 4 to 6. Figure 4 shows how the oxygen concentration in the headspace of a sterile product vial varies linearly with the peak to peak amplitude of the FMS signal. Figure 5 compares frequency modulation signals from vials filled with varying amounts of moisture. The total area is proportional to the moisture partial pressure and concentration. Figure 6 shows how the moisture laser absorption signal measures the total headspace pressure in a sealed container. As described above the moisture absorption signal width is linearly proportional to the total headspace pressure. As the total pressure rises because of a leak, the absorption signal broadens proportionately because of an increase in the collision frequency between moisture molecules and other gases. In general, measurements of higher headspace pressure require higher levels of moisture in the vial headspace.

A variety of diode laser-based system configurations can accommodate process monitoring and control and/or inspection of individual containers for oxygen, moisture or vacuum. Lighthouse Instruments, Inc., of Charlottesville, Virginia provides benchtop systems for laboratory use, as well as at-line, fully automated systems for 100% monitoring, control and

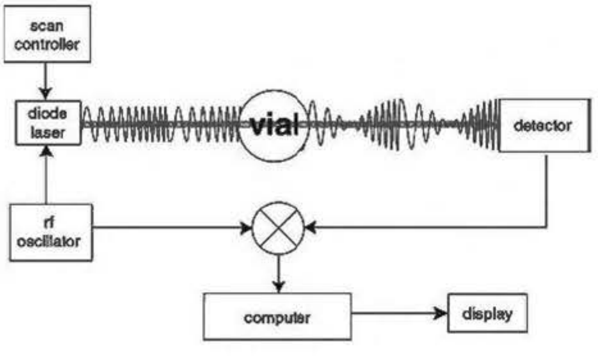


Figure 3 A schematic diagram of the frequency modulation spectroscopy technique. The frequency modulated diode laser output is converted to an amplitude modulation after passing through a gas sample, which absorbs at a particular wavelength. The amplitude modulation is proportional to gas concentration and can be phase sensitively detected. *Source*: Courtesy of Lighthouse Instruments, Inc., Charlottesville, Virginia, U.S.

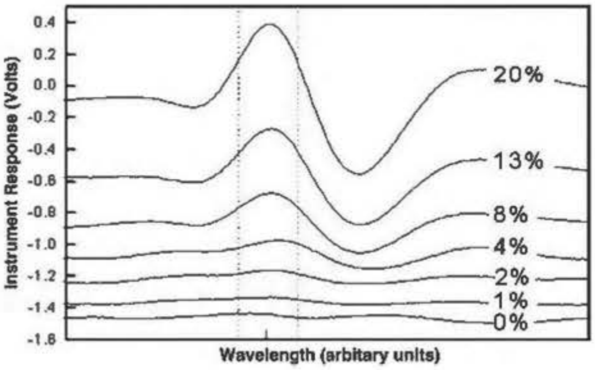


Figure 4 Frequency modulation signals from oxygen absorption. The peak to peak amplitude of each spectrum is proportional to oxygen concentration. *Source*: Courtesy of Lighthouse Instruments, Inc., Charlottesville, Virginia, U.S.

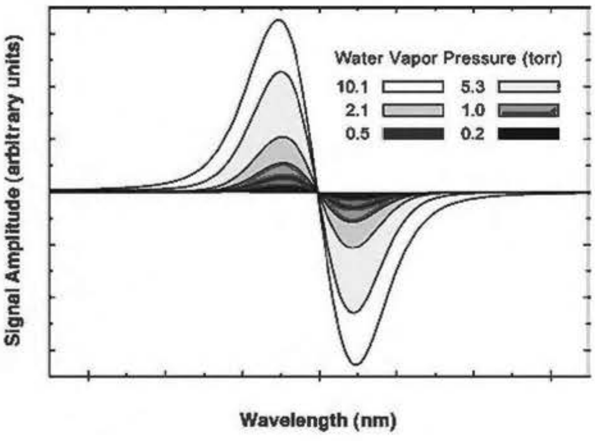


Figure 5 Frequency modulation signals from moisture absorption using 10 mL vials filled with certified amounts of moisture. Since the absorption strength of water vapor is 1000× stronger than oxygen in the near infrared, the total area of the absorption profile can be used to determine water vapor concentration. In these scans, the total area is proportional to the moisture partial pressure and concentration. *Source*: Courtesy of Lighthouse Instruments, Inc., Charlottesville, Virginia, U.S.

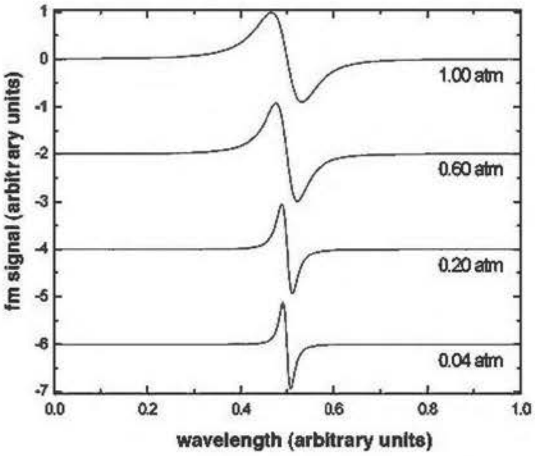


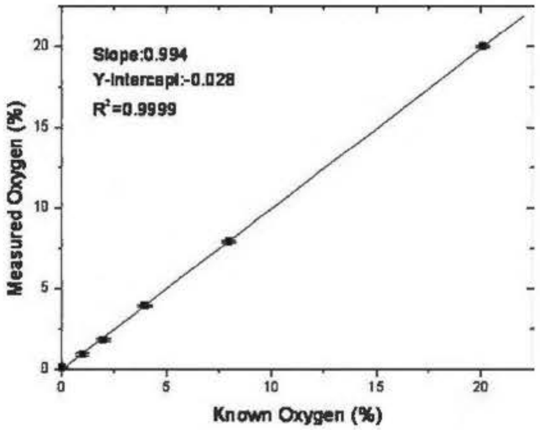
Figure 6 Frequency modulation signals due to pressure broadening of a moisture absorption signal. In principle, any molecule undergoes pressure broadening and can be used for measuring the gas pressure in a sealed container. These scans show how the absorption signal broadens as the total gas pressure increases from full vacuum to an intermediate pressure and finally to atmosphere. *Source*: Courtesy of Lighthouse Instruments, Inc., Charlottesville, Virginia, U.S.

inspection. Typical measurement times can be varied from 0.1 to 1 second corresponding to line speed throughput of 60 to 600 vials per minute. Maximum machine speeds will depend on the details of a particular application. Key parameters that impact maximum speed are container diameter and reject specification. Both faster speeds and smaller diameter packages increase measurement standard deviation.

Test systems are calibrated using NIST traceable standards of known gas concentration or pressure. Standards are constructed from the same containers used to package the pharmaceutical product, so that calibration represents containers identical to the test sample containers. For example, an oxygen-monitoring instrument would utilize standards of known oxygen concentration in containers of the same type and diameter as test sample containers. Datasets of standards measurements versus certified values enable calibration constant or calibration function generation. Subsequent measurements of unknown samples use this calibration information to convert measured absorption signals into meaningful values of headspace gas concentration and/or gas pressure. System measurement performance (method validation) is demonstrated by repeatedly testing a set of gas or pressure standards, evaluating the data following guidance in the U.S. Pharmacopeia, General Information <1225> for accuracy, precision, linearity and limit of detection (29). Figure 7 illustrates system performance data generated from 100 measurements of NIST oxygen concentration standards.

FMS offers invaluable insight for monitoring and controlling aseptic manufacturing processes. Oxygen sensitive products typically require an inert gas headspace, and lyophilized products often require either vacuum or inert gas headspace. Vial package systems, typically used for such products, cannot guarantee maintenance of inert gas or vacuum content post stoppering, prior to capping. Variations in component dimension, elastomer lubrication, gas flushing, stopper insertion, even handling, are only some of the factors that may influence the outcome. Upstream processing controls and monitors give some assurance of success, but a strong likelihood exists that some small percentage of the lot will not meet specifications. Destructive testing for either oxygen content or vacuum level using other off-line test methods is costly in terms of loss of product, and cannot provide timely information to correct a manufacturing deviation. And such test results cannot differentiate between a random glitch in the process versus system-wide failure. In contrast, FMS can be incorporated at-line for 100% automatic headspace content testing. Thus, FMS provides real-time headspace verification, enabling every unit not meeting specifications to be culled.

By testing sealed product some time post packaging, FMS technology can also verify container closure integrity, or absence of leakage. In the case of product sealed with an inert gas overlay, leakage of oxygen into the container will be a function of diffusive flow, driven by



Accuracy	0.2%
Precision	±0.08%
Linearity (R^2)	0.9999
Limit of detection	0.3%

Figure 7 Frequency modulation spectroscopy method linearity for oxygen measurement in a 10 mL vial. *Source*: Courtesy of Lighthouse Instruments, Inc., Charlottesville, Virginia, U.S.

Table 2 Time for Oxygen to Diffuse into a 10 mL Vial Container Through Holes 2 and 5 μm in Nominal Diameter

Predicted rise in package oxygen content		Time to reach predicted oxygen levels	
Partial pressure (atm)	Oxygen concentration (% atm)	5 μm hole (days)	2 μm hole (days)
0	0	0	0
0.005	0.5	<1	4
0.01	1	1	8
0.02	2	3	17
0.04	4	6	36
0.08	8	13	81

Note: Initial oxygen partial pressure is 0 torr. The defect length is assumed to be 0.1 mm. *Source*: Courtesy of Lighthouse Instruments, Inc., Charlottesville, Virginia, U.S.

Table 3 Predicted Vacuum Loss in a Leaking 10 mL Vial, Fully Evacuated Prior to Stoppering and Capping

	Package headspace pressure assuming stated leak size and laminar flow kinetics (torr)	
Time post package closing	5 μm diameter leak	2 μm diameter leak
0 min	0	0
1 min	13	2.4
5 min	63	12
10 min	126	24
60 min	756	144
5 hr	760	720
8 hr	760	760

Note: Laminar flow kinetics were modeled assuming a leak path length of 1.5 mm and air viscosity of 1.8×10^{7} Pa-sec.

the greater oxygen partial pressure outside the container. Following Fick's laws of diffusion [eqs. (2) and (3)], assuming a 10-mL vial with initial oxygen partial pressure of 0 torr, and a length of 0.1 mm separating the vial headspace and the outside environment, oxygen ingress as a function of time can be predicted (Table 2). The results show that holes ≥5 μm will permit oxygen levels to rise above 1% within one day; 2-μm holes will bring about oxygen content greater than 1% after about eight days. Caution is advised, however, when attempting to predict package integrity for longer periods according to diffusion kinetics. Over time, packages are exposed to pressure differentials from changes in altitude or weather, or even by doors opening and closing, all of which drive faster, convective flux leakage, thus complicating such projections.

Consider a second scenario, in which a 10-mL vial containing lyophilized product is stoppered under vacuum. In this case, the differential pressure between the evacuated container and the atmosphere will drive air into the package according to either molecular or laminar flow kinetics, depending on the leak path diameter, the mean free path length of the leaking gas, and the package internal pressure. Table 3 presents the projected vacuum loss that will occur for a 10-mL vial initially stoppered under full vacuum (0 torr), assuming a leak path length (vial wall thickness) of 1.5 mm, and laminar gas flow leakage. Calculations assumed laminar flow [eq. (5)] and air viscosity at 15°C (1.8×10^{-7} Pa sec). Tabulated predictions show that leakage through a hole as small as 2 μm wide is evident within several minutes after package closing; vacuum is completely lost in less than eight hours. Therefore, FMR spectroscopy is reliable and sensitive approach for verifying the integrity of every evacuated container unit both upon package sealing and as a function of stability.

Trace Gas Leak Test Methods

Leak detection by trace gas analysis is the most sensitive leak test method available. Helium is the most common trace gas used for package integrity testing, although hydrogen is also used

(30,31). Detection of helium by mass spectrometry is capable of detecting large leaks of 10^{-2} Pa m^3/sec down to ultrafine leaks as small as 10^{-11} Pa m^3/sec. Helium trace gas testing is most useful for testing leaks in the moderate to ultrafine leak range. Greatest sensitivity is possible using the vacuum mode, in which a helium-flooded sealed package is exposed to vacuum conditions while inside a closed test fixture. Mass spectrometry detects helium drawn into the fixture from the leaking package. Alternatively, the sniffer mode works by scanning the test package's exterior surfaces checking for helium leakage into the atmosphere or into a special scanning fixture. The sniffer mode can pinpoint leakage location, and is especially suited for packages that cannot tolerate test vacuum conditions. ASTM F2391-05 *Standard Test Method for Measuring Package and Seal Integrity Using Helium as the Tracer Gas* describes both vacuum mode and sniffer mode techniques (32). The ASTM method text includes P&B data demonstrating the vacuum mode's ability to differentiate between cold-form aluminum foil blister packages punctured with a needle and covered with aluminum foil laminate tape (leak rate approximately 10^{-8} cc/sec/atm), to those punctured but masked with more permeable Scotch$^{\text{TM}}$ tape (leak rate approximately 10^{-6} cc/sec/atm).

There are possible sources of error or method interferences unique to helium mass spectrometry. Background helium present in the testing environment can mask package leaks. Steps to prevent elevated helium levels in the test area include proper ventilation, remote helium cylinder location, and proper sample isolation fixturing. "Virtual" leaks resulting from helium adsorbed onto package surfaces or trapped in seal areas can be mistaken for true leakage. "Washing" surfaces free of helium using an inert gas, or drawing off adsorbed helium by adding a preliminary vacuum cycle to the leak test are sometimes used to avoid virtual leaks. Helium easily permeates through many materials, especially plastics and some elastomers. Thus, helium permeation through the test package should be known to prevent misinterpretation of results. Care should be exercised when large leaks are suspected, as helium can be quickly lost even prior to conducting the test. Finally, sensor calibration using helium reference leaks is required to ensure accurate results.

Research teams lead by Kirsch (3,8,21) and Nguygn (20) used the helium mass spectrometry vacuum mode to measure the leak rates of positive control vials prior to microbial challenge and vacuum decay leak testing. More recently, Miyako and colleagues (33) used helium mass spectrometry for verifying the integrity of a double-bag system used for holding and transporting sterile freeze-dried powder from the bulk manufacturing site to the finished product packaging site. The bulk powder was bagged in a sterilized aluminum laminate bag which was flooded with sterile-filtered helium and subsequently sealed. This inner bag was then placed in a sterile polyethylene bag which was also sealed. The helium leak test was performed by placing the double-bagged package in a vacuum chamber. After target vacuum was reached, the vacuum source was isolated from the chamber and the double-bagged package remained under vacuum for up to one hour, allowing helium leakage to occur. The chamber was then flooded with sterile-filtered nitrogen, and a sniffer probe connected to the test chamber was used to collect a gas sample for helium detection. The helium leak test was able to find pinholes present in both bags between 20 and 500 μm in size. The size of the bag and the location of the sniffer probe inserted into the test fixture influenced leak detection.

Helium leak detection is a very useful tool for container closure integrity evaluation of packages in the research and development stages of a product's life cycle. Because some expertise is required to design and conduct leak tests by helium mass spectrometry, this technology is best performed in a laboratory setting by skilled workers. When properly performed, helium mass spectrometry provides valuable information on the quantitative leak rate of a package, as well as the package's leak location.

INTEGRITY TESTING THROUGH PRODUCT LIFE CYCLE STAGES

Changing Demands Through the Life Cycle

The scope of leak tests performed may change as a product moves through the various life cycle phases of product development, marketed product manufacturing, and marketed product stability (34). Package design and development involving seal characterization and optimization demand the most package integrity support, and may in some cases, require multiple leak tests for verifying different performance criteria of individual seals. Once the package system and the assembly processes are well defined and controlled, leak tests used to support manufacturing practices may be able to focus on detecting larger leaks resulting from defective components or poor assembly.

For example, highly sensitive and quantititative helium mass spectrometry tests can be quite useful when characterizing a vial package system during package design and development. Helium leak test methods readily detect leaks at or below liquid leakage cutoff specifications. However, helium tracer tests take time to perform, are destructive to the package, may miss larger defects, and require considerable operator expertise, making this approach impractical during routine manufacturing. At the manufacturing stage, more rapid, nondestructive vacuum decay leak tests or electrical conductivity tests may make more sense for identifying leaks resulting from damage or misassembly.

While gas tracer or vacuum decay leak test methods are generically used for many container closure systems, other test methods are more product package specific. For example, electrical conductivity leak detection rapidly detects defects in liquid-filled glass or plastic packages, and is most useful in production environments for testing entire lots. Frequency modulation spectroscopy is ideally suited for testing vial package systems intended to maintain a low-oxygen or low-pressure headspace. This method is very rapid, highly sensitive, and nondestructive making it useful throughout all product life cycle phases, from research through 100% on-line production lot testing.

Integrity as a Function of Product Stability

Regulatory agencies around the world either imply or require product container closure system integrity verification as a function of stability to support new product market applications and to provide on-going postmarket product quality data. The U.S. FDA has issued several Guidances to Industry on this topic, discussed below.

The U.S. FDA Guidance of 1999 regarding container and closure systems for packaging human drugs and biologics (35) indicates the need for all pharmaceutical packaging to be suitable for its intended use. One aspect of suitability is protection the ability of the container closure system "to provide the dosage form with adequate protection from factors (e.g., temperature, light) that can cause degradation in the quality of that dosage form over its shelf life." Common causes of degradation linked to package integrity cited in this Guidance include loss of solvent, exposure to reactive gases (e.g., oxygen), absorption of water vapor, microbial contamination, and contamination by filth. Package suitability verification provided in any new product submission must therefore include package integrity study results. As stated in the Guidance, "... the ultimate proof of suitability of the container-closure system and the packaging process is established by full shelf life stability studies." And later, "Stability testing of the drug product should be conducted using the container-closure systems provided in the application ... The container-closure system should be monitored for signs of instability. Where appropriate, an evaluation of the packaging system should be included in the stability protocol." Thus, integrity testing as part of stability protocols is strongly encouraged.

The U.S. FDA Guidance for Industry describing sterilization process validation submission documentation directly communicates the need to demonstrate the ability of a container closure system to maintain the integrity of its microbial barrier, and, hence, the sterility of a drug product through its shelf life (36).

More recently, an FDA Guidance for Industry addresses the issue of integrity as part of pre- and postapproval stability protocols for sterile biological products, human and animal drugs, including investigational and bulk drugs (37). As noted, manufacturers of drugs and biologics purporting to be sterile must test each lot or batch prior to release to ensure that the product conforms to sterility requirements. While stability testing must provide evidence on how the quality of a substance or product varies with time and under specific storage conditions. Stability protocols must therefore include a method(s) that supports the continued capability of containers to maintain sterility. Sterility testing satisfies this requirement; however, this newer Guidance acknowledges practical and scientific limitations for the sterility testing approach. Therefore, this Guidance allows the substitution of other integrity tests in stability protocols according to the information and recommendations spelled out.

The FDA Guidance of 2008 does not suggest specific test methods and acceptance criteria, nor does the agency provide comprehensive lists of tests. Instead, good scientific principles are recommended, taking into consideration the container closure system, product formulations, and, where applicable, routes of administration. The Guidance states, "Any validated container and closure system integrity test method should be acceptable provided the method uses analytical detection techniques appropriate to the method and is compatible with the specific product being tested. Innovative methodology is encouraged. Information submitted to the agency should detail what the test method evaluates and how it is applicable to microbial integrity. A test method is adequately validated if it has been proven through scientifically accepted studies to be capable of detecting a breach in container and closure system integrity." The selected integrity test should be "conducted annually and at expiry, or as otherwise required by applicable regulations." Both physicochemical and microbiological challenge methods are mentioned, but the onus for proper test method selection and validation lies with the product manufacturer.

Integrity as a Function of Distribution and Use

A complete package development program should include package integrity tests performed in conjunction with distribution and end-user handling challenges. Ship testing, whether simulated in a laboratory or performed in the field, provides much more meaningful data if packages are integrity tested before and after exposure to the distribution conditions. Otherwise, it becomes difficult to ascribe package damage discovered at the end of a study to the distribution challenge. Therefore, a nondestructive leak test method is best able to detect damaged product both before and after shipping.

Use testing provides valuable insight into the functionality and integrity of packages placed in the hands of the end-user. Studies comparing package use by subjects provided with careful product package usage instructions to those given no direction provide interesting and practical information that can help in final package optimization and product literature preparation. End-user populations should vary in age, sex, education, and skill level as appropriate. This is especially important for products intended for homecare administration, or for use by the elderly or physically impaired.

Production Lot Integrity Testing: 100% Vs. Statistical Process Control

The 2008 revision to Annex 1 of the European Union Good Manufacturing Practices (GMPs) for sterile products states that "Containers closed by fusion, e.g., glass or plastic ampoules should be subject to 100% integrity testing. Samples of other containers should be checked for integrity according to appropriate procedures" (38). Additionally, "Containers sealed under vacuum should be tested for maintenance of that vacuum after an appropriate, pre-determined period." Concerning stoppered vials, "Vials with missing or displaced stoppers should be rejected prior to capping." Another reference to integrity testing in the EU GMPs states: "Filled containers of parenteral products should be inspected individually for extraneous contamination or other defects." Direction is given for human inspection, and "where other methods of inspection are used, the process should be validated and the performance of the equipment checked at intervals."

The 2004 U.S. FDA Sterile Drug Products Aseptic Processing GMPs delineate similar standards (39). Referring to inspection of container closure systems, "Any damaged or defective units should be detected, and removed, during inspection of the final sealed product. Safeguards should be implemented to strictly preclude shipment of product that may lack container-closure integrity and lead to nonsterility. Equipment suitability problems or incoming container or closure deficiencies can cause loss of container-closure system integrity. For example, failure to detect vials fractured by faulty machinery as well as by mishandling of bulk finished stock has led to drug recalls. If damage that is not readily detected leads to loss of container-closure integrity, improved procedures should be rapidly implemented to prevent and detect such defects." Appendix 2 *Blow Fill Seal Technology* states the following: "Container closure defects can be a major problem in control of a BFS operation. It is critical that the operation be designed and set-up to uniformly manufacture integral units. As a final measure, the inspection of each unit of a batch should include a reliable, sensitive, final product examination that is capable of identifying defective units (e.g., *leakers*). Significant defects due to heat or mechanical problems, such as wall thickness, container or closure interface deficiencies, poorly formed closures, or other deviations should be investigated in accordance with §§ 211.100 and 211.192."

USP <1207> *Sterile Product Packaging Integrity Evaluation* discusses the issue of 100% testing versus sample testing. This general information chapter emphasizes that control of critical production processes is paramount to integrity assurance, regardless of the integrity testing approach used (34).

To summarize, mandates to leak test every product package unit released for market currently exist only for glass and plastic BFS containers. Still, the pharmaceutical manufacturer is responsible if defective, leaking containers of any type enter the marketplace. Component quality and manufacturing process control are keys to ensuring integral packaged product, but experience says that defects still occur even under the best circumstances. For this reason, it is sensible to integrity test every production lot at least on a statistical sampling basis. Upon finding leaking packages, further lot testing and a full investigation to determine and correct the cause of the defect and to eliminate other defective units are called for. As leak test methods become available for rapid and nondestructive detection of leaks in various product package systems, it is logical to expect their implementation will become standard practice.

TEST METHOD SELECTION

Integrity test method selection is based on many factors largely addressed elsewhere in this chapter. The following brief listing summarizes major selection criteria, along with a few examples.

1. Package design and construction. Rigid, nonporous packages best tolerate test methods requiring vacuum or pressure challenge conditions, such as dye ingress tests, vacuum decay tests, or the helium mass spectroscopy vacuum mode test. Flexible packages tested by such methods require special tooling to restrict significant package expansion that may damage seals or negatively influence test method sensitivity. Packages with a porous component, such as a Tyvek lidded tray, can be tested by vacuum decay as long as a test chamber fixture or other means is used to mask the porous lidding material. Packages made of permeable materials, for example, plastics or elastomers, may not accommodate trace gas testing using gases such as helium. Electrical conductivity leak detection is able to find defects in liquid-filled packages if the liquid is more conductive than the package material.
2. Seal type and location. Package seal type and location can influence test method selection. For example, ophthalmic dropper bottles have two main seals: the dropper-tip/bottle-neck valve seal and the dropper-tip/screw-cap seal. Both seals are hidden from view under a screw-thread cap making it impossible to inspect for evidence of liquid leakage at the actual seal locations. Thus, a whole-package test able to detect gas leakage, such as vacuum decay, makes more sense in this case. On the other hand, a translucent plastic bag is easily inspected for evidence of dye migration through heat sealed areas. Electrical conductivity leak detection is an excellent choice when checking physically accessible locations at higher risk for leaks, such as the seal tip end of a plastic BFS ampoule. If a seal relies strictly on a tortuous path or the quality of a porous barrier material, then microbial challenge testing may prove necessary.
3. Critical leakage rate. Seals made to prevent liquid leakage and microbial ingress require less stringent leak rate criteria than seals meant to prevent loss of vacuum or inert gas. When verifying absence of leaks ≥ 5 μm in a nonporous, rigid package to minimize risk of liquid loss and/or microbial ingress, viable options include electrical conductivity, vacuum decay, and liquid tracer tests, assuming appropriate method optimization. Frequency modulation spectroscopy is very appropriate for headspace content verification of clear or translucent packages, both upon initial sealing and over product shelf life. With appropriate fixturing and instrumentation, helium mass spectrometry is able to quantitatively measure package leaks ranging from 10^{-2} down to 10^{-11} Pa m^3/sec. However, such trace gas methods are perhaps most useful when detecting leaks not easily found with other leak test methods, namely, below about 10^{-5} Pa m^3/sec.

4. Product life cycle phase. Tests to prove a package's most critical leakage rate of concern are commonly performed during package design and development phases. Early research may also include a wide variety of tests to satisfy particular study objectives. Once package components and assembly are optimally defined, fewer test methods may be implemented to verify absence of larger, random defects or package misassembly. For example, early development of a vial package for a liquid formulation may incorporate helium mass spectrometry to verify the critical leak rate specification; a dye ingress test as a visual aid for finding package defects; and a vacuum decay test for supporting distribution and stability studies. Later in production, an on-line electrical conductivity test may check for package defects or improper assembly.
5. Regulatory and validation requirements. Region- or country-specific regulatory requirements influence leak test method selection. A parenteral product approval to market application often includes microbial challenge test data, along with sterility tests performed as a function of product stability. However, this trend is changing. A nonmicrobial method may successfully substitute for microbial challenge tests, or replace the sterility test performed through product expiry, if strong scientific rationale and validation data supporting the alternative method are provided. A study correlating the sensitivity of the alternative method to a microbial ingress test is helpful; such comparison may be theoretical or practical. Regardless, it is important to use validated test methods to support a product approval to market application or marketed product lot release. It is not adequate simply to follow an internationally recognized ISO, ASTM or compendial method. (ASTM methods typically include P&B statements based on round robin studies. These data provide a useful starting point for test method development and validation.) Even these methods require validation studies specific to the product package system, the test equipment and the test method parameters. Validation should include verification of method robustness, reliability, accuracy and range of leak sizes detected (sensitivity). Therefore, ease of method validation is also a factor in test method selection.
6. Cost versus benefit. The costs of package integrity test methods range from a few thousand to a several hundred thousand dollars, depending on the test method and its implementation. The least expensive tests include dye, liquid tracer, and microbial challenge tests, and are therefore often preferred. However, these probabilistic tests require the destruction of large test sample populations to generate the most reliable data. Conducting such tests expends resources of time, staff, equipment, and space. Human inspection processes for detecting dye or microbial ingress are especially costly, and results are prone to error. Numerous other challenges face microbial challenge test methods, as discussed in section "Test Method Validation."

   Sometimes a given test method may vary in expense as a function of the equipment manufacturer and the method's manner of application. For example, vacuum decay leak testers come as single-chamber, manually operated test systems costing tens of thousands of dollars, or as multichamber, rotary, 100% on-line systems costing hundreds of thousands of dollars, or more. The single-chamber manual systems are not well-suited for 100% testing of large lots, but they are less costly, easier to validate, and are capable of detecting smaller leaks. Each vacuum decay equipment manufacturer uses a different methodology for detecting leakage pressure rise, which then influences the validation approach and related costs. Which test system and manufacturer is most appropriate depends on many factors, including the product, the pharmaceutical manufacturer's philosophy, the nature and size of the leaks anticipated, and the quality control systems in place for incoming package components and product manufacture. Regardless, some significant investment in integrity test method selection, validation and implementation should be expected.

TEST METHOD VALIDATION

Package integrity test methods should be validated for robustness, reliability, accuracy, and range of leak sizes detected. Quantitative analytical methodology routinely relies on these test method validation concepts. But in the case of parenteral product package physicochemical leak tests, often some assessment of the method's sensitivity to risk of microbial ingress is presumed, whether on the basis of scientific rationale or on the basis of actual laboratory studies.

Leak Test Sensitivity by Direct Comparison with Microbial Challenge Tests

How physicochemical integrity tests compare with microbial ingress tests is a topic frequently explored in publications from the food, pharmaceutical and medical device industries and academia. Generally, a population of both good and defective package units tested by both microbial ingress and the alternative container closure integrity method provide a direct comparison of the two approaches. The studies summarized below provide interesting insight on how to perform direct comparison studies, and perhaps, whether such comparisons are warranted.

About 20 years ago, the author and a team of researchers compared gas leak rates with liquid and microbial ingress from vial packages (5,40). Vials were made of stainless steel, electropolished to ensure exceptionally smooth sealing surfaces. Disc-shaped closures made of various elastomers, either uncoated or laminated with a variety of fluorocarbon- or propylene-based polymeric materials, were capped onto the metal vials at various seal forces. Test packages were mounted onto a manifold enabling them to be internally pressurized with filtered nitrogen. Package leak rates were determined by pressurizing the manifold-vial test system to target pressure, then monitoring the system's pressure drop over time. Measured gas flow rates ranged from 10^{-3} to 10^{-7} Pa m^3/sec, at 3 pounds per square inch gauge differential pressure test conditions. For the comparative microbial challenge test, each sterilized, manifold-mounted vial was filled with a suspension of *P. aeruginosa* ($\geq 3 \times 10^8$ CFUs/mL). The vial packages were submerged closure-end-down in sterile saline while being internally pressurized via the manifold. Microbial leakage into the saline was determined using a filter plate count method. In like manner, the liquid leakage test was performed by filling the vials with an aqueous solution of copper sulfate, and testing for copper ion presence in distilled water collection fluid by atomic absorption. No packages of gas leak rates less than 10^{-5} Pa m^3/sec demonstrated microbial or liquid tracer leakage. Interestingly, liquid passage occurred for every package exhibiting gas leakage at or above this rate limit, while microbial leakage only occurred sporadically, with the number of colony forming units moving across the seal bearing no relation to the gas flow rate.

In the 1990s, a team led by Lee Kirsch at the University of Iowa correlated helium leak flow rate from glass vial packages to microbial ingress and liquid leakage (8). Positive controls were made by imbedding glass micropipettes of various nominal diameters (0.1 10 μm) into the walls of glass vials. Vial package leakage was quantified by flooding open vials with helium just prior to stoppering and capping, then testing the packages using helium mass spectrometry according to the vacuum mode method. Microbial and liquid leakage through these same leak paths was determined by first filling each vial with sterile saline lactose broth. Broth-filled packages were immersed in a 60°C water bath for one hour, followed by immersion in a 25°C saline lactose broth, spiked with magnesium ion trace element, for another hour to allow the vial content's temperature to equilibrate to 25°C. The purpose of this procedure was to eliminate airlocks in the leak path. Next, the bath was spiked with 10^8 to 10^{10} viable *B. diminuta* and *E. coli* organisms/mL, and the vials continued to be immersed for 24 hours at 35°C. Post 13 days of incubation, vials were inspected for evidence of microbial growth, and vial contents were assayed for presence of magnesium tracer using atomic absorption spectroscopy.

Initially, the University of Iowa researchers only reported microbial ingress data for those test packages confirmed to contain magnesium; units failing to demonstrate a liquid pathway were eliminated from the analysis. Given these criteria, the probability of microbial ingress was near 100% at helium leak rates of about $10^{-1.9}$ std cm^3/sec (sccs), which was equivalent to about an 8-μm nominal diameter leak. An 80% probability of ingress corresponded to a leak rate of about $10^{-2.5}$ sccs (about 5 μm), and a 50% probability of ingress corresponded to a leak rate of about $10^{-3.7}$ sccs (about 0.7 μm). The likelihood of microbial failure at leak rates $\leq 10^{-5}$ sccs was remote; of the 66 test units with leak rates less than $10^{-4.5}$ sccs, only three failed the microbial ingress challenge.

Later, Kirsch used this same body of research to explore the relationship between liquid leakage verified by magnesium tracer and the likelihood of microbial ingress (6). He concluded that both liquid leakage and microbial ingress are probabilistic occurrences. For any given leak, liquid passage was more likely to occur than microbial ingress. However, even at relatively large gas leak rates greater than 10^{-4} sccs liquid leakage at times failed to occur. Microbial ingress only occurred when liquid leakage was also present, but liquid leakage did not guarantee microbial ingress. Thus, it was concluded that microbial ingress through a leak sized at $<10^{-2}$ sccs requires liquid penetration through the leak path. And liquid leakage likely depends on variables such as liquid surface tension, defect diameter, leak morphology, leak surface conditions, environmental contaminants blocking the leak, and procedural technique.

Burrell et al. compared an ISO dye ingress method with a liquid immersion microbial challenge integrity test using vial packages (13). Positive controls were created by inserting polyimide-coated glass microtubes ranging in internal diameter from 2 to 75 μm through the elastomeric closures of 5-mL vial packages. Vials were challenged with dye solution (1% FD&C Red No. 40% and 0.25% sodium dodecyl sulfate) following procedures described in ISO 8362-2 Annex C (41). Exceptions to the ISO procedure included use of red dye, rather than methylene blue, and analysis by spectrophotometry, rather than by visual inspection. Challenge conditions included package immersion in dye solution for 30 minutes at 22 in Hg (75 kPa) vacuum, followed by rapid vacuum release and 30 minutes of dye immersion at ambient pressure. There was no attempt to eliminate airlocks in the microtubes. The microbial challenge test used positive and negative control packages, filled with saline lactose broth and immersed in an *E. coli* suspension ($\geq 10^8$ CFUs/mL), challenged according to the same ISO procedure. Results showed the dye ingress test and the microbial challenge test were equally sensitive. Dye and microbial ingress occurred in at least half the units with microtubes 10 μm in diameter. No leakage of any kind was detected in packages with smaller defects (2 and 5 μm). All units of microtubes ≥20 μm demonstrated dye leakage and microbial ingress. Therefore, the ISO dye ingress method was equally sensitive to a microbial challenge test performed according to identical challenge conditions.

Keller and team published an interesting study in 2006, further exploring the relationship between critical leak size and package sterility (7). Leaking package models were created using nickel microtubes, 7 mm long, with inner diameters of 2, 5, 7, 10, 20, and 50 μm, each placed through the elastomeric septa of a small glass cell encased in a glass water jacket. Negative controls utilized solid tubes. Sterilized test cells filled with nutrient broth were placed in an aerosol chamber with tube-end down to ensure liquid broth contact with the microtube opening. Motile *P. fragi* microorganisms were aerosolized to establish a concentration of approximately 10^6 CFUs/cm^3 during the 30-minute come-up period; static conditions followed for an additional 5 minutes. Post exposure incubation continued for 72 hours at 25°C. Test cell media turbidity was indicative of microbial growth. Special ports added to each test cell enabled the simulated packages to be exposed to various controlled pressure/vacuum/temperature conditions during the biochallenge. A randomized block design allowed independent measurement of each test variable's influence on test package sterility. Considering all test variables, results showed microbial ingress can occur through microtubes as small as 5 μm in diameter; 2-μm tubes and negative controls showed no growth in any case. Test conditions that promoted broth flow into or through the tubes correlated to higher risk of microbial ingress; the greater likelihood for liquid flow, the greater the sterility loss risk. For instance, static conditions in which no differential pressure was applied only triggered microbial ingress through two of nine tubes sized 50 μm wide. Factors that promote product liquid flow and therefore increase risk of packaged product sterility loss include defect size, liquid product surface tension and the pressures imposed on the package during processing, distribution and storage.

In conclusion, all studies described illustrate the probabilistic nature of microbial ingress through package defects. Microbial challenge tests require carefully designed and conducted procedures using relatively large test sample populations to support convincing conclusions. Numerous studies have attempted to pinpoint the critical leak size that corresponds to risk of product sterility loss. Results vary, with some studies implicating leaks as small as 0.2 μm, while others imply leak paths 10 μm and larger. Regardless, and perhaps most importantly, all research shows that liquid presence in the smallest defects is required for microbes to enter. Therefore, it seems logical that industry should move away from directly correlating physicochemical leak tests to microbial challenge tests, to examining the leak test method's ability to detect defects capable of liquid passage a less stochastic and more easily verified parameter.

Leak Test Sensitivity by Indirect Comparison with Microbial Challenge Tests

Literature studies describe indirect means of correlating physicochemical leak tests to risk of microbial ingress. In two publications, vacuum decay leak tests results were compared with helium trace gas detection by mass spectroscopy. Previously, the helium mass spec method had been judged against a microbial ingress test using the same test sample population type; thus establishing an indirect relationship between vacuum decay test results to risk of microbial ingress (20,21).

Another indirect comparison approach, explained under test method *"Frequency Modulation Spectroscopy,"* is based entirely on gas leak rate predictions through a theoretical defect into an evacuated vial package. In the example cited, laminar gas flow theory was used to predict the pressure rise in 10-mL vial packages, initially sealed under vacuum, with leaks 2 and 5 μm wide. The text noted that as long as the actual vial package in question maintains an internal pressure at or below leaking package predictions, then no leaks of that equivalent size are present.

The works described in the previous subsection, "Leak Test Sensitivity by Direct Comparison with Microbial Challenge Tests," suggest that the presence of liquid in or moving through a leak path provides a better indication of the risk to package sterility afforded by the defect than a biological challenge test performed under the same test conditions. In fact, without liquid presence, microbial ingress through very small defects less than about 10 μm in nominal diameter appears improbable. With liquid presence or passage, sterility loss risk increases significantly. Therefore, a leak test reliably able to detect liquid passage can be indirectly assumed as good as, or better than, a microbial challenge test performed under the same test conditions.

Leak Test Sensitivity Based on Leak Rate Standards

Leak test method sensitivity may also be determined quantitatively using calibrated reference leak standards. Calibrated physical leaks are designed to deliver gas at a known flow rate. There are many types of standard leaks, falling into two main categories: (*i*) reservoir leaks that contain their own tracer gas supply and (*ii*) nonreservoir leaks that rely on tracer gas addition during testing. Calibrated gas leaks perform by one of two methods. Either the leakage rate depends on the permeation of specified materials by certain gases, or an orifice is present allowing specified gas flow rates under prescribed differential pressure conditions. Some leak test instruments, for example, helium mass spectrometry, incorporate internal reference standards to verify test system functionality.

Other leak test instruments that rely on air movement for leak detection, for example, vacuum decay testers, may utilize a calibrated variable rate flowmeter or a fixed size orifice to artificially introduce leakage into a test chamber during equipment qualification or start-up.

Whenever possible, leak test instrument performance should be challenged using such calibrated standards. The *Nondestructive Testing Handbook*, Volume 1 *Leak Testing* (42) is an excellent resource for precautions and limitations regarding calibrated leak usage. While calibrated leak standards provide valuable instrument functionality and sensitivity information, it is still important to challenge a leak test method using known positive and negative control package samples.

Positive Control Test Samples

Defect Types

Leak test sensitivity verification is not complete without a demonstration of successful leak detection using a randomized population of negative and positive control test samples. A positive control is a known-leaking test package. A common misconception is that a media-filled package used for a growth promotion check in a microbial challenge test is equivalent to a positive control test sample. A growth promotion test only proves that the packaged media can support microbial growth; it does not prove that bacteria would or could actually enter the package. Another false perception is that a calibration standard, such as a calibrated airflow introduced into a vacuum decay leak test chamber, satisfies the need for a positive control test. Certainly, such a test is important as it correlates equipment response (pressure rise) to a known challenge (airflow rate). However, it does not prove that the method can detect leaks of various sizes or types at various locations on the package.

Simple ways commonly used to create positive control test samples involve inserting microtubes or needles through package walls, placing wires or film between sealing surfaces, or adhering thin metal plates with microholes over package surface openings. These types of defects are inexpensive, simple to create, and give a quick assessment of a leak test's capabilities. Because microtubes, microholes and needles have fixed diameters, test results infer detectable leak path sizes. On the other hand, such positive controls do not truly represent defects most likely to occur in actual product packages. Liquid or microbial migration around or through an item foreign to the package (e.g., needle, film, microhole, or microtube) may be very different from leakage through an actual defect located in or between package components.

A study by Morrical and associates illustrated this very point, by comparing helium leakage and microbial ingress through two types of defects in glass vial packages (43). One defect type consisted of a laser-drilled microhole in a thin metal plate mounted on a holed-stopper, capped on each test vial. Microholes ranged in diameter from 0.5 to 15 μm. The other leak type was a copper wire placed along the sealing surface between the elastomeric closure and the glass vial. Wire thicknesses ranged from 10 to 120 μm. Helium trace gas leakage was detected using mass spectrometry. The microbial challenge test included a suspension of *S. marcescens* ($\geq 10^8$ CFUs/mL). Challenge conditions consisted of one hour at 0.4 bar vacuum followed by one hour at 0.4 bar overpressure. Both test methods showed different leakage behavior for the two positive control types. Helium leak rates through the microholes matched theoretical predictions for gas moving through an orifice, whereas helium flow rates through the wired samples displayed complex, less predictable, gas flow dynamics. Microbial ingress occurred in at least a portion of the samples with microholes ≥ 4 μm (helium leakage rate $\geq 6.1 \times 10^{-3}$ mbar L/sec), while units with holes ≤ 2 μm ($\leq 1.4 \times 10^{-3}$ mbar L/sec) saw no microbial leakage. Microbial challenge results for hand-capped vials with wire defects demonstrated microbial leakage for wire diameters >20 μm (helium leakage rate $\geq 2.2 \times 10^{-5}$ mbar L/sec).

Whenever possible, positive control test samples should incorporate defects simulating actual leaks likely to occur. For example, typical vial package defects may include glass cracks or breaks (Fig. 8), misaligned or misshapen closures, and poorly crimped seals. Therefore, a laser-drilled hole in a glass vial wall could simulate vial breakage. Including defects positioned above and below the liquid fill level is important if the leak test method's performance is a function of liquid or gas presence in the leak path. Scoring the vial finish might represent another type of glass defect (Fig. 8). Removing slices along a closure's sealing surface, or loosely capping seals can replicate closure and seal defects, respectively. Pouch or bag positive control samples might include pinholes, open seals, channeled or wrinkled seals, weak seals, "burned" seals, and seals with trapped product inclusions. Ophthalmic dropper bottle positive controls could include loose caps, missing or poorly inserted dropper tips, defective tips or caps, and pinholes in the bottle.

With the exception of laser-drilled hole defects, the positive controls described will not necessarily provide information about the exact sizes of detectable leaks, but they will help define detectable leak locations and types. Risks inherent in this approach include the possibility that the leak test would not find all nonhole positive controls, and that the irregularities in defects' shapes or sizes may not permit statistically sound method reliability and sensitivity assessments. Nevertheless, including such positive controls in leak test method feasibility and optimization studies can provide invaluable information on the method's capabilities. Knowing this may give insight into ways of limiting the occurrence of actual defects not readily found by the chosen leak test method.

Defect Sizes

Published studies using microtubes or other artificial means to create leaks have unfortunately resulted in an expectation that all leak test methods need to detect defects as small as 0.2 μm in diameter, otherwise, the test method cannot compare to microbial ingress.

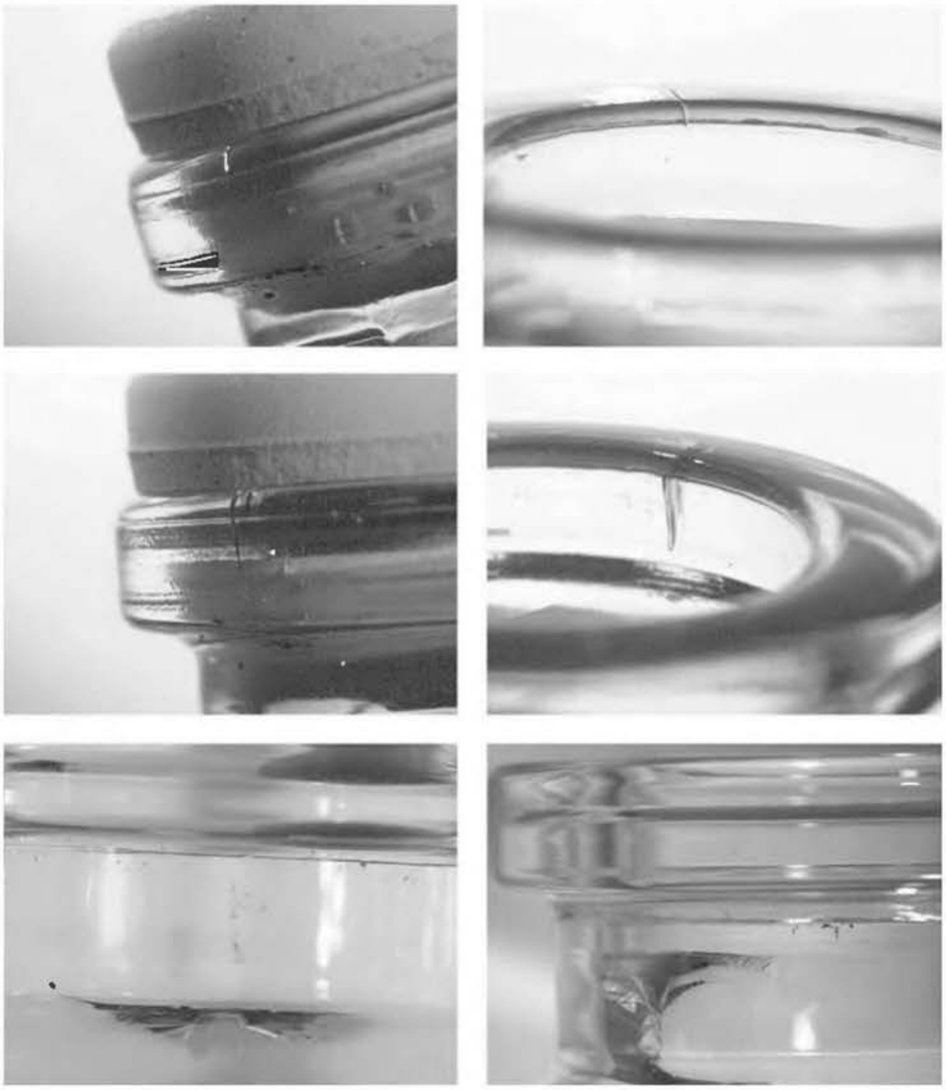


Figure 8 Defects found in glass vials. *Top row*: Line over defect likely created during vial manufacturing process. *Middle row*: Crack in vial finish likely created during vial manufacturing or distribution to end user. *Bottom row*: Crack in vial shoulder (*left*) and vial neck (*right*) likely created at the end user manufacturing site. *Source*: Anonymous upon request.

The first problem with this premise is creating defects 0.2 μm in size. Experience says naturally occurring leaks in packages below a few micrometers wide are extremely rare, if they occur at all. Also, defects are not hole-shaped, but are complex tortuous paths. Even artificial laser-drilled holes through the walls of glass vials or syringes are really a convoluted matrix of capillaries and chambers (Fig. 9). Companies that laser drill holes certify their size by comparing the rate of pressurized gas flow through each hole with flow rates through standard orifices in thin metal plates. Generally, the smallest possible laser-drilled holes through small volume glass or plastic containers range from about 3 to 5 μm in nominal

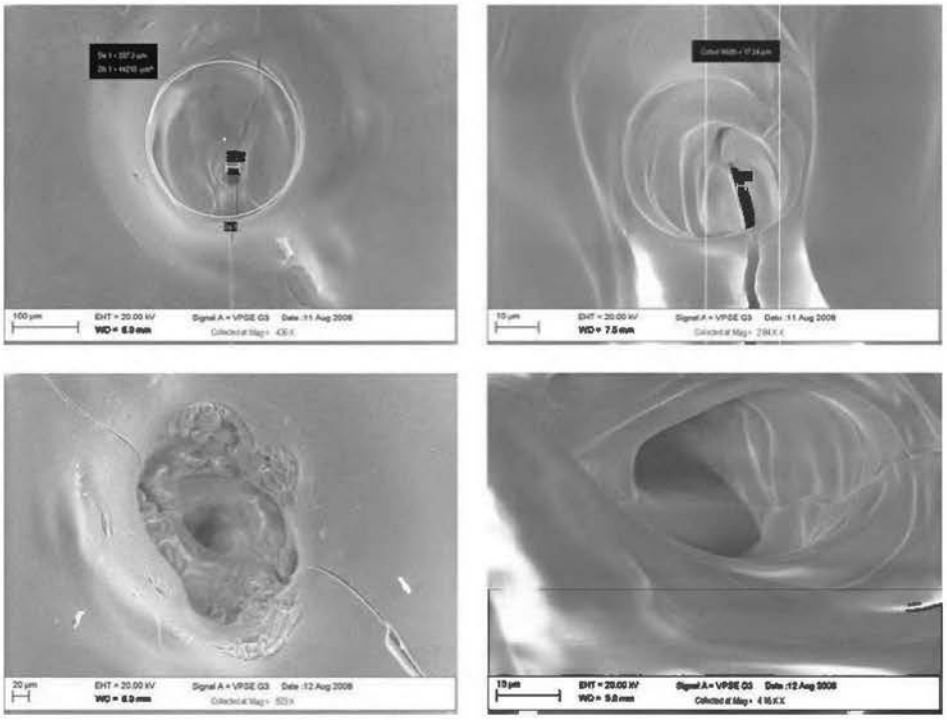


Figure 9 Scanning electron micrographs of laser drilled holes through the glass barrels of 1 mL prefillable syringes. Each hole was nominally sized by comparing the rate of pressurized airflow passing through each hole with the flow rate through precisely formed, standard holes in thin metal plates. Nominal hole sizes are 10 μm (*top row*) and 15 μm (*bottom row*). *Source*: Reprinted from Amgen, Inc., Thousand Oaks, California, U.S.

diameter; smaller holes are difficult to make and readily clog. The smallest feasible holes through flexible laminates or films may vary from about 2 to 10 μm in diameter depending on the packaging material. Without a way of creating and sustaining holes sized below these practical limits, positive control test samples with smaller defects are not possible.

The other factor complicating this requirement is even typical microbial ingress tests cannot find 0.2-μm defects. Microbial ingress tests by Kirsch et al. (8) only found submicron-sized defects in a very small fraction of samples, under extreme challenge conditions, after meticulous measures to eliminate leak path plugs and airlocks. The risk of microbial ingress rose significantly for defects >1 μm, exceeding 80% probability for defects about 5 μm, and approached 100% probability for 8-μm defects. All defects considered in this analysis where those already confirmed as allowing liquid passage. In the absence of liquid passage, no microbial ingress occurred with any size defect (6). Research by Burrell et al. linked microtube defects ≥10 μm to a significant chance of dye and microbial ingress (13), while Keller's work using aerosolized microorganisms implicated microtube leaks ≥5 μm (7). Morrical detected microbial ingress in a portion of vial packages topped with thin metal plates having microholes ≥4 μm (43).

Therefore, positive control leaks should be as small as reasonably possible, given the type of package, the package dimensions, and the materials of construction. Parenteral product package positive control test units used for checking the lower limit of sensitivity of physicochemical leak test methods generally include defects ≥5 μm in diameter. Positive control sample populations should include larger defects as well as smallest defects, to represent the full range of anticipated leak sizes.

CONCLUSION

Container closure integrity is an easy concept to grasp. Simply put, packages must contain and protect their contents, preventing leakage in or out. However, the many parenteral product types and package integrity requirements make leak test method selection and leakage measurement anything but a simple process. First, leakage is not a straightforward, yes-or-no phenomenon. All package seals have the potential to leak gases to some extent; therefore, an understanding of leakage flux and critical leak rate specifications is necessary. When selecting leak test methods, microbial challenge tests are the traditional choice, despite their cumbersome application and demonstrated lack of reliability and sensitivity. Alternative physicochemical leak test methods are increasingly popular, including dye or liquid tracer methods, vacuum decay leak tests, electrical conductivity tests, frequency modulation spectroscopy, and trace gas detection. Each approach has unique advantages and disadvantages. Often more than one test may be necessary to provide full product support through all product life cycle phases. Any test selected must be appropriately developed, optimized and validated prior to use. Tools necessary for this process include calibrated reference leak standards, and positive and negative control test samples. The technique used to create leaks in positive control packages, and the size of these leaks, are significant factors in leak test sensitivity interpretation. Traditionally, final definition of leak test sensitivity requires some indirect or direct correlation to risk of sterility loss. Debate continues on the best approach to address this expectation, but mounting evidence supports a shift away from microbial ingress direct comparison studies. In summary, the last three decades have seen parenteral product container closure integrity move from a package testing afterthought to a major feature of product quality assessment. This evolution will likely drive the development of more reliable and sensitive package integrity test methods for future parenteral products.

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Index

ABELCET, 151
Absorption, distribution, metabolism, and excretion (ADME), 30, 37
  physicochemical properties of drugs and, 30, 37
Absorption, drug, 38 39
  effect of physiological factors on, 41
Acceptable quality level (AQL) sampling plans, 294, 295 296, 300
Active pharmaceutical ingredient (API), 58
Actives, pharmaceutical, requirements for
  in depot delivery, 160
ADA. *See* Antidrug antibody (ADA)
ADD Vantage® system, 318
Adhesives, 360
ADME. *See* Absorption, distribution, metabolism, and excretion (ADME)
Adsorption, 70
  of proteins, 225 226
AFFF. *See* Asymmetric flow field flow fractionation (aFFF)
Age, effect on pharmacokinetics
  absorption, 41
  distribution, 42
  metabolism, 41
Aggregates
  characterization of, 214 215
  formation of, 215
  protein, 214 215
  separation methods, 215
  types of, 215
Aggregation
  defined, 214
  Freeze thaw induced, 215
  in proteins
    covalent linkage, 226 227
    noncovalent linkage, 226
AIDS
  parenteral drug delivery and, 8
Airflow meters, 367
Airlines, 302
Albumin
  cross linked polymers, 179
  stabilizer, 237
Aliphatic PCs, 309
Alkaline ions, removal of, 291
Alkaline residues, 301
Allergic conjunctivitis, 264
Alternative equilibria, for solubilization, 134, 146 151
  complexation and association, 146 148
[Alternative equilibria, for solubilization]
  emulsions, 149 150
  liposome, 150 151
  micellar, 148 149
Aluminum cations (Al^{3+}), 289
Aluminum seal capping, 362
Aluminum seal parenteral package, 359
AmBisome, 151
American Society for Testing Materials (ASTM), 125
Amino acids sequence
  composition analysis, 200
  liabilities determination in, 198
  structure of, protein and, 199 200
Ammonium sulfate treatment, 301
Amorphous solids, solubility and, 141
Amphipathic substance, 91
Amphotericin B
  drug solubility and, 135
  liposomes, 151
Ampoule container system, advantages, 295
Ampoules, 294 295
Analog strategies, solubility and, 145
Analytical ultracentrifugation (AUC), 215, 216
ANN. *See* Artificial neural networks (ANN)
Anterior chamber injection, 20, 21. *See also* Intraocular injection
Anterior segment of eye (topical), delivery to, 258
  conjunctival and scleral absorption, 260
  corneal absorption, 259
  elimination from anterior segment of eye, 260
  nasolacrimal drainage, 259
Antiallergy agents, 264
Antibody induction, biotherapeutics and, 45. *See also* Biotherapeutics
Antidrug antibody (ADA), 45
  classification of
    binding (BAbs), 46
    neutralizing (NAbs), 46
Antigen presenting cells (APCs), 45
Antiglaucoma agents, 264 265
Antiglaucoma drugs, 264 265
Antihistamines, 264
Anti infective agents, 262
Anti inflammatory agents, 262, 264
Antimicrobial effectiveness testing (AET), 275
Antimicrobial preservatives
  in formulation development, 99 100
    compatibility of, 100
    effectiveness of, 100
    minimum inhibitory concentrations of, 99

Antioxidants
  in formulation, 63
  in formulation development, 96 99, 122
    mechanism of action of, 96
Antipsychotics, in depot delivery systems, 158 159
APCs. *See* Antigen presenting cells (APCs)
API. *See* Active pharmaceutical ingredient (API)
Apparent permeability (P_{app}), 34
AQL based sampling plans, 300
AQUAFAC. *See* Aqueous functional group activity coefficients (AQUAFAC) model
Aqueous functional group activity coefficients (AQUAFAC) model, 143
Aqueous humor, 257
Aqueous vehicle, 91
Artificial neural networks (ANN), 143
Ash percentage, 336
Asparagine deamidation, 211 212
ASTM. *See* American Society for Testing Materials (ASTM)
ASTM E 438, 292
ASTM E 438 92, 292
ASTM F2391 05 standard, 375
Asymmetric flow field flow fractionation (aFFF), 215, 216
ATR. *See* Attenuated total reflectance (ATR) accessory
Atrigel technology, 175
ATryn, 222
Attenuated total reflectance (ATR) accessory, 206
A type phase solubility profiles, 147
AUC. *See* Analytical ultracentrifugation (AUC)

BAbs. *See* Binding antibodyies (BAbs)
Banbury type mixer, 324
Beer Lambert law, 200
Benzalkonium chloride (BAK), 265
β elimination, in proteins, 230
Binding antibodyies (BAbs), 46
Biodegradable polymeric depot delivery systems
  lipid based systems in, 180 181
  natural and synthetic polymers in, 177 179
  PLA in, 167
  PLGA in, 167 177
Biological considerations
  in formulation development, 114 115
"2710 Biological Finish" standard, 298
Biopharmaceutical considerations
  in formulation development, 77
Biopharmaceutics, 30
  and routes of administration, 258
Biotherapeutic bioequivalence, 51 53
Biotherapeutics, 30
  antibody induction and
    T cell dependent pathway, 45
    T cell independent pathway, 45
  characterization of
    formulation, 195, 198 199
    structural, regulatory agencies on, 194 195
  classes of, 194
  examples of, 196 197
[Biotherapeutics]
  immunogencity of, 45 51
    chemistry manufacturing and control (CMC) and, 47 48
    container/closure system, 49 50
    impact of process and formulation, 48 50
    product related impurities and degradation products, 48 49
  potency determination in, 195
  protein based. *See* Proteins
  structure of , CMC and, 194 195
Bisphenol A PC, 309
Blemishes, 302 303
Blisters, 302
Blood retina barrier, 258
Blowback feature, of glass, 298
Blow molding process, 296 298, 297, 310
Blown film extrusion, 310
Bonding forces
  solubility and, 79 81
Boron oxide (B_2O_3), 288, 291
Borosilicate glasses, 290 291
  formulations, 296
Bottle, 295 298
Bowman's membrane, 256
Brain tumor resection, depot formulations for, 158
Brittle failure, in glass, 302
"Brittle" material, 301
Browning reaction, 231
Bruch's membrane, 258, 261
BUBBLE FREE FILLING®, 317
Buffers
  in formulation development, 93 96
  in protein formulations, 235
Building block, of silicate glasses, 288
Bulking agents, in protein formulations, 238 239
Bulk staked needle syringes, 299
"Bulk" staked needle syringes, 299
Buminate®, 318
BYDUREON, 158
BYETTA, 158

CAD. *See* Collisionally activated dissociation (CAD)
Calibrated gas leaks, 382
Camera based inspection systems, 296
Capillary electrophoresis (CE), 223
Capillary gel electrophoresis (CGE), 214
Capillary IEF, 213
Capronor, biodegradable polymer, 177
Carbohydrate solutions, 123, 124
Cartridges, glass, 298 299, 299
CD. *See* Circular dichroism (CD) spectroscopy; Cyclodextrins (CD), complexation
CE. *See* Capillary electrophoresis (CE)
Cellulosic biodegradable polymers, 179
CGE. *See* Capillary gel electrophoresis (CGE)
CGMPs. *See* Current good manufacturing practices (cGMPs)
Charge heterogeneity, 211 213
Charge profiling, 211

Charge transfer (CT) complex, 138
Chemical bonding techniques, 360
Chemical dealkalization effect, 301
Chemically bonded seals, 360 361
Chemical resistance, of container surface, 300 301
Chemisorption, 70
Chemistry manufacturing and control (CMC), 194
  immunogenicity and, 47 48
Chitosan polymer, in gelling system, 179
Choriocapillaris, 258
Choroid, 258
Circular dichroism (CD)
  spectroscopy, for proteins, 205 206
  for T_m, 224
Classical immune response, 46
Closure/vial seal integrity, 340 341
Clumping, 346
CMC. *See* Chemistry, manufacturing, and controls (CMC); Critical micelle concentration (CMC)
Coated closures, 335 336
Coated vial stoppers, 335
Cochleates, in depot delivery systems, 180 181
Cocrystals, 144
Codman 3000 implantable pump, 182
Cold denaturation, Freeze thaw induced aggregation *vs.*, 215
Collapse temperature (T_c), 109
Collisionally activated dissociation (CAD), 204
Color break bands, 294
Commercial container glasses, 287 288
Common ion effect, 59, 60
Compatibility studies. *See also* Support studies in formulation development
  with diluents and IV administration sets, 115
  of excipients, 68 69, 70
  with manufacturing equipment surfaces, 115
  with membrane filters, 115 116
  with packaging components, 115
Compendial classifications of glass compositions, 291 292
Complexant systems
  solubility in, 61
Complexation, 146 148
Compression set, 337
Concentration time profile, 37 38
Conjunctiva, 257
Conjunctival absorption, 260
Connectivity index, 140
CONSTA, 159
Contact lens care solutions and rewetting drops, 277 278
Container closure system
  choice of
    for drug substance and products, 240 242
  immunogenicity and, 49 50
  for parenteral drug delivery, 3
Container components
  on formulation, 77, 117
Convective flow of gases, 362
COP/COC packaging systems, for parenteral delivery, 311
COP/COC syringes, 316
Copolymers (COC), 311
Cord, 302
Coring, 341
Cornea, 256 257
Corneal absorption, 259
Corticosteroids, 262, 264
Cosolvents
  dielectric constants and, 136
  solubility enhancement by, 145
  solubility in, 60, 85
  stability and, 85 87
Cremophor EL, surfactant, 149, 153
Critical micelle concentration (CMC), 61, 88, 90, 149
  effect of temperature on, 89
Crystallinity, 65 66
Crystallization
  controlled. *See* Milling
  for drug powder preparation, 107
Crystal® technology, 311 312
Current good manufacturing practices (cGMPs), 1
Cyclic olefin copolymer (COC), 306
Cyclic olefin polymers (COP), 306, 311
Cyclic olefins, 306
  in parenteral drug delivery, 306 308
Cyclodextrins (CD), 264
  complexation, 146 148
  solubility and, 91
Cysteine, oxidation of, 229
CZ plastic vials, 312
CZ resin, 310
CZ syringe systems, 316
CZ vials, 312
  crystallinity of mannitol in, 313

Daikyo Crystal Zenith®, 306
Daikyo CZ syringe system, 315
Danner process, 293 294
DC. *See* Dielectric constant (DC)
3 D crystalline quartz, 289
Dealkalization processes, 292
  excessive, impact, 301
Deamidation
  of asparagine, 211 212
  in proteins, 227 228
Defects, of glass, 302 303
Degradations
  pathways, in proteins, 225 231
  physical and chemical, in biotherapeutics, 198 199
Delamination effect, 301
Denaturation, in proteins, 225
Dental anesthetics, 298
DepoFoam product, 180
Depot delivery systems
  advantages of, 158 160
  antipsychotics in, 158 159
  biodegradable polymers in, 167 181
  compliance rates and, 158 159
  defined, 158

[Depot delivery systems]
  development, 182 185
    IVIVC and, 184
    in vitro method, 183
    in vivo method, 183 184
    in manufacturing process and scalability, 184
    strategy and economics, 185
    in terminal sterilization, 184
  drawbacks to, 158, 159 160
  future of, 185 187
  history and types of, 160
  implantable device based and nondegradable, 181 182
  new applications for, 186 187
  pharmaceutical actives for, 160
  plasma concentration in, 158, 159
  regulatory considerations, 184 185
  suspension and oily vehicle, 160 166
Depot formulations, 1
Depyrogenation, 291, 299
Descemet's membrane, 256
Design of experiments (DOE) approach
  for formulation development, 240
Dextran, cross linked, 178
Diabetes type II, depot formulations for, 158
Diamond crystal, 206
Dielectric constant (DC)
  solubility and, 79 80
  of solvent, 136
Di(2 ethylhexyl) phthalate (DEHP) plasticizers, 309
Die trimming, of elastomeric closures, 325
Differential scanning calorimetry (DSC), 58
  for T_m, 217 218, 224
Diffusion, 33 34
Diffusion coefficient (D), 361
Diketopiperazine formation, in proteins, 231
Dimethyl ester of terephthalic acid (DMT), 309
Diode laser based system configurations, 371
Diode laser devices, for testing, 371
Dipole ion interaction
  solubility and, 81
Diprivan emulsion, 150
Disc shaped closures, 380
Disposable infusion pumps, 27
Disposable technology, 318 319
Dissolution, 35 36
Distribution, 39 40
  effect of physiological factors on, 42
Disulfide bond determination
  in IgG, 208
  for proteins, 207 208
Disulfide bond formation and scrambling, 226
Disulfide exchange, in proteins, 227
DLS. *See* Dynamic light scattering (DLS) measures
DOE. *See* Design of experiments (DOE) approach
Dosing error
  parenteral drug delivery and, 1
Double ended needle, 298
Dried forms, drug, 107
Drug degradation, 61 64
  modes of
    hydrolysis, 62 63
    oxidation, 63
    photolysis, 63 64
  protein drug, 72 73
Drug delivery, parenteral. *See* Parenteral drug delivery
Drug delivery systems, enhanced, 271
  cationic liquid formulations, 272 273
  high viscosity liquid formulations, 272
  in situ gelation, 272
  mucoadhesive liquid formulations, 272
  prolonged delivery polymeric systems, 273 274
  transporter mediated drug delivery, 274
Drug interactions
  with formulation components, 67 69
  with manufacturing surfaces, 70
  with packaging components, 69 70
Drug release, enhanced control, 186
Drug substances
  physicochemical properties of
    material properties, 65 67
    molecular properties, 57 65
Dry eye syndrome, 264
Dry eye treatments, 264
DSC. *See* Differential scanning calorimetry (DSC)
Dual transducer test system, 367
Duplex® Drug Delivery System, 318
Duran®, 291
Durin, 175
Duros, implant, 182
Dynamic light scattering (DLS) measures, 215, 217

Economics, depot delivery systems development and, 185
Edman degradation, N terminal sequencing by, 200 201
EDTA. *See* Ethylenediaminetetraacetic acid (EDTA)
EG monomer, 309
"Elastic" material, 301 302
Elastomeric parenteral closures
  aging factor, 345 346
  classification of manufacturing environment and environmental controls, 326
  coated, 335 336
  common types and designs, 327 332
  compatibility factors, 345
  die trimming process, 325
  ISO standard on biological material properties, 345
  mixing and preforming, 324 325
  molding process, 325
  packaging, 326, 352 354
  properties of pharmaceutical, 336 346
  quality control and quality assurance, 354 356
  raw materials, 324
  standards, 356 357
  sterilization, 350 352
  using rubber compounds, 332 335
  USP standards, 344

[Elastomeric parenteral closures]
  washing and siliconization, 346 350
  washing process, 325 326
Elastomeric septum, 298
Electrical conductivity testing, 370
Electrolyte solutions, 122 124
Electrospray ionization (ESI), 202
Electrostatic interaction
  for protein adsorption, 226
Elucidation of Structure and Impurities section, of CMC, 194
EMEA. *See* European Medicines Agency (EMEA)
Emulsions, 71 72, 149 150
  formulations, 71, 271
  parenteral, 104 105
Enzymes
  for N linked glycans, 211
  proteolytic, 202
Epidural administration, 17 18
  Tuohy needle and, 18
Epidural space, 17
  analgesia and, 17
Episclera, 257
Equilibrium
  defined, 134
  subsaturation state and, 134, 135
  supersaturation state and, 134, 135
  thermodynamic solubility and, 134 135
Equipment, parenteral drug delivery
  types of
    infusion pumps, 24 25
    infusion regulators, 24
    syringe pumps, 25 26
ESI. *See* Electrospray ionization (ESI)
Ethanol, on solubilization, 152 153
Ethylenediaminetetraacetic acid (EDTA), 98
Ethylene glycol (EG), 309
Ethylene oxide sterilization, 309, 351 352
Ethylvinyl acetate (EVA), 319
Ethyvinyl alcohol (EVOH), 319
EU compendia, 367
European Medicines Agency (EMEA), 194, 320
Eutectic temperature (T_{eu})
  determination of, 109
  freeze drying process and, 109
Excel®, 317
Excipients
  compatibility studies of, 68 69, 70
  new, introduction of, 186
  in parenteral formulations, 68
  in protein formulations, 244
  safety of, 50
  in suspensions, stabilization, 165 166
Excretion, drugs, 40 41
33 expansion borosilicate glass container, 302
"90 expansion" soda lime glass, 302
Exposure response relationship, drugs, 42 44
  pharmacokinetic analysis, 42 43
  pharmacokinetic pharmacodynamic (PK/PD) analysis, 43
Extracellular fluids, 119
  characteristics of, 120
Extractables, 320
  according to pharmacopeial methods, 339 340
Extrusion blow molding, 310
Extrusion process of plastic, 310

Far ultraviolet (far UV) lamp, CD and, 205 206
Far UV, Far ultraviolet (far UV) lamp
FDA. *See* U.S. Food and Drug Administration (FDA)
FDA's guidance document, 320
Feeding behavior of closures, 346
Ferric oxide (Fe_2O_3), 289, 291
Fibrous tunic
  conjunctiva, 257
  cornea, 256 257
  sclera, 257
Fick's laws of diffusion, 33 34, 361, 363, 374
Filling, in vials, 311
Filter extractability testing, 116. *See also* Compatibility studies
Finished product inspection, 355 356
Flange diameter, 327
Flange forming process, 299
Flange thickness, 328
Flat sheet extrusion, 310
Flocculated system, in suspension formulation, 163
Fluorescence spectroscopy, for protein, 208 210
Fluoropolymer coated closures, 335
Fluoropolymer coatings, 335
Fluoropolymers, 335
Flurotec®, 315
Folded structure, in biotherapeutics
  hotspot and, 198
Food and Drug Administration (FDA) inactive ingredients database, 265
Form selection, drug, 64 65
  polymorphism, 65
  salt form, 64 65
Formulation
  compatibility of, 3
  container components on, 117
  effect of development process on, 117 118
  photostability of, 116 117
  stability evaluation of, 117
Formulation characterization, 195, 198 199
  hot spots, determination of, 198
  methods, 216 219
  physical and chemical degradations, 198 199
Formulation components
  drug interactions with, 67 69
Formulation development. *See also* Large volume parenteral (LVP); Small volume injections
  biopharmaceutical considerations in, 77
  goal of, 76
  influence of route of administration in, 77 78
  for large volume injections, 118 127
  for small volume injections, 76 118
    support studies, 114 117
    template for, 113

[Formulation development]
  solubility and, 77
  stability and, 77
Formulation parameters
  evaluation of
    buffers, 235
    bulking agents, 238 239
    ionic strength/salt concentration, 235 236
    pH, 233 235
    preservatives, 239
    protein concentration, 232 233
    stabilizers, 236 238
    tonicity modifiers, 238
Formulations, protein
  for antibodies, 246 248
  buffers, 235
  bulking agents, 238 239
  development, DOE approach, 240
  development process in industry, general, 231 232
  excipients, 244
  for human growth hormone, 244, 245
  ionic strength, 235 236
  of marketed protein products, 244 249
  parameters. *See* Formulation parameters
  pH, 233 235
  protein concentration in, 232 233
  salt concentration, 235 236
  screening and optimization, strategies for, 239 240
  stabilizers, 236 238
  tonicity modifiers, 238
Fourier transform infrared (FTIR) spectroscopy
  amide I band in polypeptides, frequencies of, 207
  ATR accessory, 207
  for peptides and proteins, 206 207
  water interference and, 206
Fragmentation, in proteins, 229
Freeze dried pharmaceuticals
  advantages of, 108
  characteristics of, 109
  packaging of, 111
Freeze drying, 312
Freeze drying closure, 328 329
Freeze drying process, 108 112
  eutectic temperature and, 109 111
  phases of, 109 112
    freezing of solution, 109
    primary drying, 109 111
    secondary drying, 111
  temperature and pressure curves for phases of, 112
Freeze thaw induced aggregation, 215
Freezing of solution
  in freeze drying cycle, 109
Frequency modulated spectroscopy (FMS), 371 374
Freundlich Ostwald equation, 141
FTIR. *See* Fourier transform infrared (FTIR) spectroscopy
Functional packaging, 352 353

Galaxy® Drug Delivery System, 318
GAPs. *See* Good aseptic practices (GAPs)
Gaskets, 331
Gas permeability, 337
Gauge transducer, 367
Gel
  forming, polymers, 179
  systems, PLGA and, 174 175
GelSite polymer, in gelling system, 179
General solubility equation (GSE) model, for solubility prediction, 143
Germanium (GeO_2), 288
Glass, defined, 287
Glass ampoules, 360
Glass ampoule sealing process, 360
Glass Container Manufacturer's Institute (GCMI), 298
Glass containers, as packaging materials, 287
Glass flaws, 302
Glass flow rate, control of, 293
Glass forming process, for pen cartridge, 299
Glass formulation, backbone of, 288
Glass fracture analysis, 303
Glass Packaging Institute (GPI), 298
Glassy silicon dioxide, 288
Glaucoma, 264 265
GLIADEL wafer, 158, 178
Gliding behavior of syringe plungers, 343
Glycine, bulking agent, 238
Glycosylation
  immunogenicity and, 48
  of proteins, 210 211
Goblet cells, 254, 255
Gold nano particle staining techniques, 312
Good aseptic practices (GAPs), 1
GPI 2710 standard, 298
Gravity infusion, 22 23
GSE. *See* General solubility equation (GSE) model
"Guidelines for the photostability testing of new drug substances and products," 116

Halobutyl elastomers, 333 334
Hardness, 336
H1 blockers, 264
Helium leak detection, 375 376
Helium leak rates, 363, 383
Helium mass spectrometry, 375
Helium trace gas testing, 375
Hemolysis, 114
Henderson Hasselbach equation, 36, 138
Henderson Hasselbach relationship, 82, 83, 94 95
Heparins, 314
Hepatitis C
  parenteral drug delivery and, 8
High density polyethylene (HDPE), 306, 308
High molecular weight species (HMWS), 226
High performance liquid chromatography (HPLC), 82, 201, 211, 213
High voltage leak detection (HVLD), 370
Higuchi, Takeru, 30

HMWS. *See* High molecular weight species (HMWS)
Hospira's ADD Vantage® system, 318
Host cell contaminants
  immunogenicity and, 48
Hot spots
  determination, in formulation characterization, 198
  linear sequence *vs.* folded structure in, 198
  protein and peptide degradation, 198, 199
HPLC. *See* High performance liquid chromatography (HPLC)
HSA. *See* Human serum albumin (HSA)
Human growth hormone
  formulations for, 244, 245
  methionines in, 230
Human serum albumin (HSA), 244
Hydrates, solubility of, 140 141
Hydrogen bonding
  solubility and, 81
  solute and, 139
Hydrolysis
  drug degradation and, 62 63
  of polypeptide, 200
Hydron, implant, 181
Hydrophilic polymers, on solubilization, 152, 153
Hydrophobic interaction
  for protein adsorption, 226
Hydroscopic product packages, 364
Hydrotropes, for water solubility, 147
Hydroxyls, 300
Hygroscopic lyophilized products, packaging of, 361
Hypersensitivity, cremophor EL and, 149

ICE. *See* Imaged capillary electrophoresis (iCE)
ICH. *See* International Committee on Harmonization (ICH)
"ICH process," 117
ID. *See* Intradermal (ID) administration
Ideal vial based system, 312
IEC. *See* Ion exchange chromatography (IEC)
IEF. *See* Isoelectric focusing (IEF)
IgG. *See* Immunoglobulins (IgG)
IM. *See* Intramuscular (IM) administration
Imaged capillary electrophoresis (iCE), 213
Imaging technology, for protein particulates, 217
Immunogencity
  biotherapeutics and, 45 51
    chemistry manufacturing and control (CMC) aspects of, 457 458
    container/closure system, 49 50
    glycosylation and, 48
    host cell contaminants and, 48
    product related impurities and degradation products for, 48 49
    therapeutic effect, 46 47
  evaluation and prediction of, 50 51
Immunoglobulins (IgG)
  charge heterogeneity and, 212
  disulfides in, 208
[Immunoglobulins (IgG)]
  hot spots in, 198
  MS of, 203 204
Implanon, implant, 181
Infections
  parenteral drug delivery and, 8
Inflammation, 262
Infusion pumps, 24 25. *See also* Parenteral drug delivery
  disposable, 27
Infusion regulators, 24. *See also* Parenteral drug delivery
Injectability
  of suspensions, 70
Injectable suspension
  formulation, 103
Injection
  large volume intravenous (IV) solutions, 76
  small volume injections, 76 118
Injection molded items, 310
Injection molding process, 310
Injection molding technology, 325
Inner filter effect, 208 209
Inorganic silicates, 287
Insertion behavior of stoppers, 346
Inspection procedures, 367
Insulin pumps, 182
Insulin therapy, 298
Integrated container closure system, 298
International Committee on Harmonization (ICH), 194
International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, 117
Interstitial fluids, 119
Intra abdominal administration, 19 20
  bowel puncture and, 20
Intra arterial administration, 14 15
  radiopaque substances and, 14
Intra articular administration, 2, 18 19
Intracardiac injection, 20
  risk factors, 20
Intracellular fluids, 119
Intracisternal administration, 15
  neurological injury and, 15
Intradermal (ID) administration, 2, 14
  mistakes in, 14
Intramuscular (IM) administration, 1, 11 12
  sites for, 12
Intraocular injection, 20 21
  types of, 20
Intraocular irrigation solutions, 274
Intraperitoneal route. *See* Intra abdominal administration
Intrathecal administration, 16 17
  baclofen and, 16
Intrathecal pump, 16
Intravascular fluids, 119
Intravenous immunoglobulin (IVIG) therapy
  toxicity, 50

Intravenous (IV) administration, 1, 9 11
  complications of, 10
  indications for, 10
  sets, 310
Intravenous (IV) minibags, 317
Intravenous (IV) nutritional therapy, 118
Intravenous (IV) spikes, 310
Intraventricular administration, 15 16
Intravitreal implants, 265
Intravitreal injections, 20, 21, 262. *See also* Intraocular injection
In vitro in vivo correlations (IVIVC)
  in depot delivery systems, 184
In vitro method development
  in depot delivery systems, 183
In vitro release methods, accelerated, 183 184
  in depot delivery systems, 183 184
Ion exchange chromatography (IEC), 213, 223
Ionic strength, in protein formulations, 235 236
Ionic surfactants, 88
  critical micelle concentration and, 89
Ionization, and solubility, 141 142
Ionization constant (K_a), 32, 58, 138
Ionization (pK_a), drugs, 32
Irradiation sterilization, 351
ISO dye ingress method, 381
Isoelectric focusing (IEF), 213, 223
Isoelectric point (pI), of proteins, 223
ISO international standards, 367
  ISO 3302 1, 328
  ISO 4802 1, 301
  ISO 4802 2, 301
  ISO 7886 1, 332
  ISO 8362, 298
  ISO 8362 1, 295
  ISO 8362 2, 328
  ISO 8362 4, 296
  ISO 8362 6, 329
  ISO 8536 2, 328
  ISO 8536 6, 329
  ISO 9187 1, 294
  ISO 9187 2, 295
  ISO 11040, 299
  ISO 11040 2, 331
  ISO 11040 5, 331
  ISO 11137, 351
  ISO 13926, 299
  ISO 13926 2, 331
Isomerization, in proteins, 227 228
Isoosmosity, 102
Isothermal titration calorimetry (ITC), 218
Isotonicity
  formulation development and, 78, 100, 102
ITC. *See* Isothermal titration calorimetry (ITC)
IV. *See* Intravenous (IV) administration
IVIG. *See* Intravenous immunoglobulin (IVIG) therapy
IVIVC. *See* In vitro in vivo correlations (IVIVC)

Jadelle, implant, 181
Japanese Pharmacopoeia (JP), 305

Karl Fischer method, 218
Kimax®, 291
Knots, 302
Knudsen's law for molecular flow, 363

Lacrimal fluid, 256
Lactoferrin, 255
Lamina fusca, 257
Laminar flow, 362
Large unilamellar vesicles (LUV), 105
  liposomes, 151
Large volume injections. *See* Large volume parenteral (LVP)
Large volume parenteral (LVP) packaging, 305, 317 318
Large volume parenteral (LVP) solutions, 115
  admixture considerations in, 126 127
  formulation of, 118 127
    effect of processing conditions on, 126
    packaging parameters of, 122
    pH effect on, 121
    physical parameters of, 122
    physiological parameters of, 120 121
    solubility of, 121
    vehicles for, 122
  functions of, 118 119
  stability evaluation of, 125 126
  stabilization of, 122
  stress testing in, 125
Laser drilled defects, 367, 369
Laser drilled hole defects, 383 384
Laser induced fluorescence (LIF) detection, 213
Latanoprost formulation, 265
Latent heat of fusion, 140
Latex allergy, 334
Leachables, 320, 340
Leakage flux, 361 362
Leakage units of measure, 363
LFC method test, 369
LIF. *See* Laser induced fluorescence (LIF) detection
Lighter weight syringes, 317
Linear sequence, in biotherapeutics
  hotspot and, 198
Lipid based depot delivery systems
  cochleates, 180 181
  lipid microparticles, 180
  liposomes, 180
Lipids
  in liposomes, 151
  microparticles, in depot delivery systems, 180
Lipodystrophies, 13
Lipophilic compounds, 260
Lipophilicty, of solute, 138 139
Liposomes, 150 151, 180
Liposomes in formulation development, 105 106
  formation
    mechanical dispersion method, 106
    phospholipids and, 106
Liquid chromatography (LC)
  MS and, 202
Liquid leak tests, 366

Liquid plugged leak paths, 369
Liquid tracer leak test, 366 367
L_{iso} method
  for tonicity calculation, 101 102
Liver damage, cremophor EL and, 149
Log*P*, in solubility prediction, 143
Lomefloxacin
  complexation of, 147
  pH and NaCl on solubility of, 142
Low density polyethylene (LDPE) resins, 308
Luer Lock syringes, 299
Luer tip syringe, 299
LUV. *See* Large unilamellar vesicles (LUV)
LVP. *See* Large volume parenteral (LVP)
Lyophilization, 291, 312 313. *See also* Freeze drying process
Lyophilized product, bulking agents for, 238 239
Lysozyme, 255

MAb. *See* Monoclonal antibodies (mAb)
Machineability of elastomeric closures, 346
Macroheterogeneity, 210
Maillard reaction, in proteins, 230 231
MALDI. *See* Matrix assisted laser desorption/ionization (MALDI)
Manganese oxide (MnO), 291
Mannitol, 313
  bulking agent, 238
Manufacturing and handling support studies
  in formulation development, 115 117
Manufacturing process development
  of drug substance and drug product, 242 243
Mass spectrometry (MS)
  ESI, 202
  of IgG, 203 204
  LC and, 202
  MALDI, 202
  proteolytic mapping with, 203 204
  tandem, 204 205
  TOF, 202
Material properties of, drug substance, 65 67
  appearance, 65
  crystallinity, 65 66
  thermal properties, 66 67
  vapor sorption analysis, 67
Matrix assisted laser desorption/ionization (MALDI), 202
Matrix loaded system, in polymeric systems, 181 182
Maximum entropy method, 209
MDA. *See* Medical Device Amendment (MDA)
Mechanical dispersion method
  for liposome production, 106
Mechanically fitted seals, 359 360
Medical Device Amendment (MDA), 25
Meibomian glands, 255
Meibomian secretions, 255
Melting, for microspheres, 174
Melting points, in solubility prediction, 142 143
Melting temperature (T_m)
  in protein formulations, DSC and, 218
Membrane compatibility study, 115 116. *See also* Compatibility studies
2 Mercaptobenzothiazole, 333
Metabolism, 40
  effect of physiological factors on, 41 42
Metallocene catalysis, 306
Methionine, oxidation of, 229 230
Micellar, 148 149
Miceller solubilization, mechanisms of, 88
Microbial challenge tests, 358, 365 366, 383
Microbial ingress, 365 366, 383
Microbial ingress/liquid leakage relationship, 364
Microbial membrane retention testing, 115. *See also* Compatibility studies
Microbiological state of cleanliness, 348
Microemulsions, 150
Microheterogeneity, 210 211
Microscopy, for protein particulates, 217
Microspheres, PLGA
  encapsulation technologies for, 168, 172 174
  melting and spray drying technique for, 174
  o/w emulsion technique for, 168, 172
  phase separation technique for, 173 174
  s/o/w emulsion technique for, 172 173
  $w_1/o/w_2$ emulsion technique for, 173
MICs. *See* Minimum inhibitory concentrations (MICs)
Milling, 164, 166
Minibags, 317 318
Minimum inhibitory concentrations (MICs)
  of antimicrobial preservatives, 99
MLV. *See* Multilamellar large vesicles (MLV)
MLx, 311
Modifier cations, 289
Modulus of elasticity of glass composition, 302
Moisture absorption/desorption behavior, 338
Molality, 79
Molarity, 79
Molar volume, of solutes, 140
Molded bottles, 296, 297
Molecular connectivity, 140
Molecular diffusion, drugs, 33 34
Molecular flow, 363
  leak rates, 363
Molecular properties of, drug substance, 57 65
Molecular surface area, of solute, 139 140
Molecular topology, solute and, 139
Monoclonal antibodies (mAb)
  charge heterogeneity and, 212
  disulfide bonding and, 208
  in LC/MS, 203
  for metastatic colorectal carcinoma, 208
MS. *See* Mass spectrometry (MS)
Multicomponent glass, 3 D structure of, 290
Multidose products, preservatives for, 239
Multilamellar large vesicles (MLV), liposomes, 151
Multilamellar vesicles (MLVs), 105
Multilayer bags, 319
Multilayer plastics, 309
Multiple linear regression (PLS), 143

Multivesicular liposomes
  in depot delivery system, 180
Multivesicular vesicles (MVV), 105
MVV. *See* Multivesicular vesicles (MVV)

NAbs. *See* Neutralizing antibodies (NAbs)
N acetylneuraminic acid (NANA), 48
NANA. *See* N acetylneuraminic acid (NANA)
NanoCrystal technology, 164
Nanosuspensions, 71, 106 107
  defined, 106
  developmental considerations in, 106
  for IV, advantages of, 106
  particle size distribution and, 106
Naproxen, solubilization of, 153
Nasolacrimal drainage, 259
Nasolacrimal system, 254 256
  parts of, 254
  tear fluid lipid content, 255
  tear fluid mucus layer, 255
  tear fluid osmolality, 256
  tear fluid pH and buffer capacity, 255 256
  tear fluid proteins and enzymes, 255
  tear fluid secretion and volume, 254
  tear fluid viscosity and surface tension, 256
National Intravenous Therapy Association (NITA), 119
Near infrared (NIR) spectroscopy, 218
Near ultraviolet (near UV) lamp, CD and, 205 206
Near UV. *See* Near ultraviolet (near UV) lamp
Nernst Brunner equation, 35
Network building block, for glasses, 288
Neulasta®, 316
Neuroleptics, o/w method in, 168
Neutralizing antibodies (NAbs), 46
N glycolylneuranimic acid (NGNA), 48
NGNA. *See* N glycolylneuranimic acid (NGNA)
NIR. *See* Near infrared (NIR) spectroscopy
NIST oxygen concentration standards, 373
NIST traceable standards, 373
NITA. *See* National Intravenous Therapy Association (NITA)
N linked glycosylation, 210 211
Nonaqueous vehicle, 91 92
Nonbridged oxygen atoms, 289
Nondestructive leak test method, 377
Nonionic surfactants, 87 88
  critical micelle concentration and, 89
Nonsterilized vials, 314
Nonsteroidal anti inflammatory drugs (NSAIDs), 262, 264
No pop stopper, 327
2 Norbornene, 306
Norplant, implant, 181
Noyes Whitney equation, 35
N terminal sequencing, by Edman degradation, 200 201
Nutritional solutions, 123, 124
Nutritional therapy, intravenous (IV), 118. *See also* Large volume parenteral (LVP)
Nutropin Depot, 176

OctoPlus, 178
Ocular allergic disorders, 264
Oil in water (o/w) emulsion technique
  for microspheres, 168, 172
Oils as vehicles, 91 92
Oily vehicle depot systems, 166
  suspensions and, 160 163
Ointment formulations, 268 269
OncoGel polymer system, 175
"One Point Cut" (OPC) opening system, 294 295
On line electrical conductivity test, 379
Online vacuum filling system, 317
Online vacuum stoppering system, 317
Ophthalmic delivery
  excipients for use in, 265
  formulations for, 265
Ophthalmic dropper tip bottle, 359
Ophthalmic formulations, 254
  future developments, 283
  packaging, 280
  preservation of, 274 275
  preservative free multidose devices, 280 281
  regulatory pathways for
    ophthalmic medical devices, 282
    ophthalmic new drug applications, 281 282
  stability storage and testing of, 283
  sterile manufacturing, 278 279
Ophthalmic indications and diseases, 262
Ophthalmic package production, 359
Ophthalmic solutions
  formulation, 265, 268
  viscosity of, 256
Optical distortion, 296
Osmosis, 120
Osmotically driven systems, 182
Ostwald Freundlich equation, 164
Ostwald ripening, 164
Oxidation
  of cysteine, 229
  drug degradation, 63
  of methionine, 229 230
  of tryptophan, 230
Oxidation potential
  pH effect on, 96, 97

P. fragi microorganisms, 381
PA. *See* Polyanhydrides (PA) polymers
PAB®IV containers, 318
Package seals, closure integrity tests
  ASTM F2338 09 standards, 369
  characterization and optimization, 359 361
  dye and liquid tracer method, 366 367
  electrical conductivity testing, 370
  frequency modulated spectroscopy (FMS), 371 374
  helium leak detection, 375
  100% integrity testing, 377 378
  for leakage, 361 364, 361 366
  leak test sensitivity verification, 383 385
  with microbial challenge tests, 380 382
  microbial ingress tests, 381 382, 385

[Package seals, closure integrity tests]
  NIST oxygen concentration standards, 373
  test method selection, 378 379
  test method validation, 379 385
  through product life cycle stages, 375 378
  trace gas analysis, 374 375
  U.S. FDA Guidance, 376 377
  using calibrated reference leak standards, 382
  vacuum decay leak test, 367 370
  and viscoelastic properties, 360
Pain
  at injection site, 114 115
Parenteral Drug Association (PDA), 303
Parenteral drug delivery
  advantage of, 1, 7 8
  development, historical aspects of, 3 5
  disadvantage of, 1, 8 9
  equipment, types of, 24 26
    infusion pumps, 24 25
    infusion regulators, 24
    syringe pumps, 25 26
  quality aspects of, 2 3
  routes of administration, 1 2, 9 21
    epidural, 17 18
    intra abdominal, 19 20
    intra articular, 2, 18 19
    intracardiac, 20
    intracisternal, 2, 15
    intradermal, 14
    intramuscular (IM), 11 12
    intraocular, 20 21
    intrathecal, 2, 16 17
    intravenous (IV), 9 11
    intraventricular, 15 16
    subcutaneous (SC), 12 13
  small molecule drugs, absorption in, 30 32
  techniques
    gravity infusion, 22 23
    pressure infusion, 23
Parenteral drugs
  storage and handling of, 8
Parenteral nutrition, 124 125
Parenteral products
  solubility of, 134 135
  suspensions
    formulation of, 164 166
    manufacture and control of, 166
Particle size, drug
  on physical stability, 164
  on release rate and pharmacokinetics, 164 165
  on syringeability, 164
Particulate cleanliness of elastomeric closures, 348 349
Particulates, protein, 214 215
Partition coefficient (P), 32 33, 57 58
PAT. *See* Process analytical technologies (PAT)
Patient controlled analgesia (PCA), 26 27
  advantages of, 26
  disadvantages of, 26 27
PBPK. *See* Physiology based pharmacokinetic modeling (PBPK)
PCA. *See* Patient controlled analgesia (PCA)
PC based polymers, 309
PCL. *See* Polycaprolactones (PCL) polymers
PC resin, 309
PCS. *See* Photon correlation spectroscopy (PCS)
PDR. *See* Physicians' desk reference (PDR)
Penetration forces, for elastomeric closures, 342
Penetration thickness, 328
Peptides. *See* Proteins
Permanent deformation, 337
Permeability, drugs, 34
Permeation, 361
Permeation rate constant (K_P), 361
pH
  adjustment
    cyclodextrins and, 148
    surfactants and, 151 151
  in deamidation/isomerization, 227 228
  derivation and, Henderson Hasselbalch equation, 138
  disulfide bonded aggregation and, 227
  effect on oxidation potential, 96, 97
  effect on solubility, 82 83. *See also* Buffers
  and NaCl on solubility of lomefloxacin, 142
  in protein formulations, 233 235
  protein solubility and, 224
  solubility profile, 141 142
Ph. Eur. Test for Surface Hydrolytic Resistance, 291 292, 301
Pharmaceutical development principles, proteins and, 231 244
Pharmaceutical parenteral drug delivery, 305
Pharmacokinetic pharmacodynamic analysis (PK/PD), 43
Pharmacokinetics (PK), 30, 37 41
  absorption, 38 39
  distribution, 39 40
  effect of physiological factors on, 41 42. *See also* Absorption; Distribution; Metabolism
  excretion, 40 41
  metabolism, 40
  particle size on, 164 165
Pharmacopeia, 356
Phase separation technique, for microspheres, 173 174
Phe fluorescence, 205, 208
Phenytoin, drug solubility and, 135
Phlebitis, 114
Phosphorus (P_2O_5), 288
Photodegradation, drug, 63 64
  effect of wavelength on, 64
Photolabile drugs, 116, 117
Photolysis
  drug degradation and, 63 64
Photon correlation spectroscopy (PCS), 217
Photostability, 116 117
Phototoxicity, 63 64
pH partition hypothesis, 32, 33
pH solubility profile, 59 60
Physical integrity of container, 301

Physical stability, of drug
  particle size on, 164
Physicians' desk reference (PDR), 244
Physicochemical constants, 57 58
  ionization constant, 58
  partition coefficient, 57 58
Physicochemical container closure integrity method, 358
Physicochemical properties of, small molecule drugs
  adjustable or changeable, 31 32
  effect on absorption, 30 32
  effect on pharmacokinetics, 37 41. *See also* Absorption; Distribution; Excretion; Metabolism
  intrinsic, 31
Physiological factors
  effect on pharmacokinetics, 41 42
Physiology based pharmacokinetic modeling (PBPK), 43
PK. *See* Pharmacokinetics (PK)
PK/PD. *See* Pharmacokinetic pharmacodynamic analysis (PK/PD)
PK/PD model, 43 44
PLA. *See* Poly(lactic acid) (PLA)
Plackett Burman design, 69
Plasma concentration
  in depot delivery systems, 158, 159
Plastic BFS ampoules, 360
"Plastic deformation," 301
Plasticizers, 309
Plastic molding processes, 309 310
Plastic packaging systems
  chemical compatibility, 319
  extractables and leachables, 320
  marketing considerations, 318 319
  for pharmaceutical drug products
    advances in plastics, 306 310
    large volume parenterals, 317 320
    prefillable syringe systems, 314 317
    quality and regulatory considerations, 320 321
    vials, 310 314
  protein adsorption, 319 320
  quality and regulatory considerations, 320 321
  sterile barrier integrity, 320
Plastic reconstitution, 313
Plastic resins, 312
Plastics fabrication, 309 310
Plastic syringes, 315, 317
Plastic vial adapters, 313
Plastic vial to vial transfer systems, 314
PLGA. *See* Poly(lactic co glycolic) acid (PLGA)
Plug diameter, 327
Plungers, 330 332
Plunger stopper, 300, 330
Pneumatic gas leak flow, 362
POE. *See* Polyortho esters (POE)
Poiseuille's law for laminar flow, 362
Polarity, of solvent, 136
Polarizability, of solute, 137 138
Poloxamer 407 polymer, in gelling system, 179
Polyamino acid polymers, 178 179
Polyanhydrides (PA) polymers, 178
Polybutylene terephthalate (PBT), block copolymers of, 178
Polycaprolactones (PCL) polymers, 177
Polycarbonate (PC), 309
Polydimethylsiloxane, 299
Polyethylene (PE), 308
Polyethylene terephthalate (PET), 309
Polyhydric phenols, 309
Poly isoprene compounds, 334
Poly(lactic acid) (PLA), 167
Poly(lactic co glycolic) acid (PLGA)
  encapsulation technologies for, 168, 172 174
  gel/rod systems, 174 175
  microspheres, 168, 172 174
  polymer selection and degradation, 167 168
  products, 169 171
  proteins and peptides, delivery of, 175 177
Polymeric systems, nonbiodegradable, 181 182
Polymers, 307
  bi axial orientation of, 310
  in biodegradable depot delivery systems, 167 177
  natural and synthetic biodegradable
    albumin, cross linked, 179
    cellulosic, 179
    dextran, cross linked, 178
    gel forming, 179
    polyamino acid, 178 179
    polyanhydrides, 178
    polybutylene terephthalate, 178
    polycaprolactones, 177
    polyortho esters, 178
    polyphosphoesters, 177 178
  PC based, 309
  selection and degradation, in PLGA, 167 168
  and sutures, resorbable, 167
Polymorphism, 65
Polymorphs, solubility and, 140
Polynuclear aromatics (PNAs), 333
Polyortho esters (POE), polymers, 178
Polypeptides
  FTIR frequencies of amide I band in, 207
  FTIR spectra and, 206 207
  hydrolysis of, in amino acid composition analysis, 200
Polyphosphoesters (PPE) polymers, 177 178
Polypropylene (PP), 308 309
Polysorbate 80, 90 91, 244
  surfactant, 149, 151, 153
Polysorbates, 315
Polyvinyl chloride (PVC), 309
Posterior drug delivery, 260 262
Posterior segment of eye (topical), delivery to
  intravitreal injection/implant, 262
  subconjunctival/transscleral delivery, 261 262
  topical, 260
Post thaw viability, 312
Potassium oxide, 289

Potency
  actives, requirements for, 160
  determination in biotherapeutics, 195
Powder filling, 107 108
  particle size and, 107
Powder X ray diffraction (XRD), 218 219
PPD. *See* Purified protein derivative (PPD) test
PPE. *See* Polyphosphoesters (PPE) polymers
Precipitation, of drug, 114
Prefillable syringe plunger, 331
Prefilled syringes, 299 300, 305
  global regulatory approvals in cyclic olefin polymers/cyclic olefin copolymers, 314
  market considerations, 314 315
  process considerations, 317
  protein stability improvement strategies, 315 317
Preformulation stability studies, 64
Preservatives
  adsorption of, 338 339
  for protein formulation, 239
Preservatives used in ophthalmic formulations, 275
  acids, 277
  alcohols, 277
  biguanides and polymeric biguanides, 276 277
  oxidizing agents, 277
  parabens, 277
  polyquaternary ammonium compounds, 276
  quaternary ammonium compounds, 276
Pressure infusion, 23
  infusion equipment used, criteria of, 23
Primary drying, 109 111
Process analytical technologies (PAT), 118
Prodrug approach, 259
Prodrug strategy, solubility and, 145
Profile tubing extrusion, 310
ProMaxx technology, 179
Prostaglandin analogs, 265
Protein adsorption, in CZ vials, 312
Protein aggregation, with silicone oil, 316
Protein concentration, in formulations, 232 233
Protein drugs
  chemical stability of, 72
  physical stability of, 73
Proteins. *See also* Amino acids sequence; Enzymes; Polypeptides
  aggregates and particulates, 214 215
  biotherapeutics
    physical and chemical degradations in, 198 199
  degradation pathways, 225 231
    adsorption of, 225 226
    β elimination in, 230
    deamidation in, 227 228
    denaturation in, 225
    diketopiperazine formation in, 231
    disulfide exchange in, 227
    fragmentation in, 229
    isomerization in, 227 228
    Maillard reaction in, 230 231
    non covalently linked aggregation in, 226
    oxidation in, 229 230
    racemization in, 228 229
[Proteins]
  formulation development principles
    container and closure system, 240 242
    formulation parameters evaluation, 232 239
    formulation screening and optimization, 239 240
    manufacturing process development, 242 243
    process in pharmaceutical industry, 231 232
    stability studies, 243 244
  formulations of marketed products, 244 249
  heterogeneity of, 222 223
  isoelectric point of, 223
  modifications, after translation
    charge heterogeneity in, 211 213
    glycosylation of, 210 211
    size heterogeneity in, 214 215
  molecular composition of, 222 223
  and peptides depot systems
    polymers and, 175 177
  primary structure assessment
    amino acid composition analysis for, 200
    MS for, 202 205
    N terminal sequencing for, 200 201
    proteolytic mapping for, 201 202
  secondary structure assessment
    CD spectroscopy for, 205 206
    FTIR spectroscopy for, 206 207
  solubility, 223 224
  structure of, 222 223
  tertiary structure assessment
    disulfide bond determination for, 207 208
    fluorescence spectroscopy for, 208 210
  thermal transition midpoint (T_m) in, 224
Proteolytic mapping
  with MS, 203 204
  of proteins, 201 202
Pumps
  Codman 3000 implantable, 182
  insulin, 182
  SynchroMed, 182
Purified protein derivative (PPD) test, 2
Pyrex®, 291
Pyroglutamic acid formation, 212

QbD. *See* Quality by design (QbD)
QELS. *See* Quasielastic light scattering (QELS)
QSAR. *See* Quantitative structure activity relationship (QSAR)
Quality attributes, of glass, 302 303
Quality by design (QbD)
  formulation development process and, 117
Quantitative structure activity relationship (QSAR), 139, 143
Quartz, 288, 289
Quasielastic light scattering (QELS), 217

Racemization
  in proteins, 228 229
Raman spectroscopy, 217
Raw material composition, significance in glass composition, 293

Ready for sterilization (RfS) bags, 326, 352 353
Ready to use (RtU) bags, 326, 353
Reconstitution, 312 313
REDIFLOW^{TM}, 318
Reflex tearing, 268
ReGel polymer system, 175
Release rate, of drug
  particle size on, 164 165
Resealing of an elastomeric closure, 342 343
Reservoir system, in polymeric systems, 181
Resin, 308, 312
Retinal pigment epithelium (RPE), 258
Retinal tunic, 258
Retrobulbar injection, 20, 21. *See also* Intraocular injection
Rewetting drops and contact lens care solutions, 277 278
Rexam, 311
Rigid needle shields, 331
Rigid tip caps, 331
Ring opening metathesis polymerization (ROMP) process, 306
RISPERDAL, 159
Rod systems, PLGA, 174 175
Routes, parenteral drug administration, 1 2, 9 21. *See also* Parenteral drug delivery, routes of administration
  formulation development and, 77 78
Rubber plunger, 330

Salt concentration, in protein formulations, 235 236
Salt formation, 64 65
  and solubility, 83 84, 142, 144
Salting out effect. *See* Common ion effect
SC. *See* Subcutaneous (SC) administration
Schizophrenia, depot formulations for, 158
Schlemm's canal, 257, 264 265
Schott, Otto, 290
Schott Forma Vitrum, 311
Schott TopPac$^{®}$, 311
Sclera, 257
Scleral absorption, 260
Scleral permeability, 261
Scotch^{TM} tape, 375
Screw cap closure, 359
SDS PAGE. *See* Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE)
SEC. *See* Size exclusion chromatography (SEC)
Secondary drying, 111
Secretory immunoglobin A, 255
Seeds, 302
SELCON. *See* Self consistent method (SELCON)
Selectively permeable membranes, 34
Self consistent method (SELCON), 206
Semi permeable plastic BFS ampoules, 360
Semi rigid plastic trays, 360
"Shake flask" solubility method, 82
Shelf life support
  stability studies for, long term, 243 244
Shelf storage, of prefilled syringes, 300
Silica (SiO_4), 288
Silicate glasses, 288
  families, 289 292
Silicone, use in syringe manufacturing, 315
Silicone oil, 316
  interaction, 315
Silicone oil free CZ syringes, 316
Siliconization
  of closure, 349
  of rubber closures, 326
Siliconize syringes, 316
Si O bonds, 300
SiO_4 tetrahedron, 288
Size exclusion chromatography (SEC), 215
Size heterogeneity, of proteins, 214 215
SLS. *See* Static light scattering (SLS) measures
Small molecule drugs, physicochemical properties of
  effect on absorption, 30 32
Small unilamellar vesicles (SUV), 105
  liposomes, 151
Small volume injections
  formulation of, 76 118
    added substances and, 77
    container and closure components and, 77
    template for development of, 113
    vehicle selection and, 77, 78
Small volume parenteral (SVP) packaging, 305
Smart pumps, 26
Soda lime glass, 291
Soda lime silicate glasses, 289 290
Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE)
  for size heterogeneity, 214
Solid in oil in water (s/o/w) emulsion technique
  for microspheres, 172 173
Solid phase separation, 135
Solubility, 34, 36 37
  actives, requirements for, 160
  in aqueous system, 58
  in complexant systems, 61
  cosolvent and, 60, 85
  cyclodextrins and, 91
  defined, 78, 135
  dielectric constant (DC) and, 79 80
  dipole ion interaction and, 81
  drug, steps for, 134
  effect of pH on, 82 83
  effect of temperature on, 81 82
  expression of, 79
  formulation development and, 77, 78 91, 121
  hydrogen bonding and, 81
  ionization and, 141 142
  measurement of, 82
  methods to enhance, 151 153
  modifications to
    solid state, 144 145
    solution phase, 145
  for monoacidic compounds, 36
  for monobasic compounds, 36
  in nonaqueous system, 58

[Solubility]
  of parenteral products, 134 135
  pH solubility profile, 59 60
  prediction, 142 143
  of proteins, 223 224
  salt formation and, 83 84
  solutes in. *See* Solutes
  solvents in. *See* Solvents
  surfactants and, 60 61, 87 91
  of zwitterions, 142
Solubility coefficient (S), 361
Solubility parameters, of solvent, 136 137
Solubility pH profile
  for weak acid, 83 84
  for weak base, 83 84
Solubility product (K_{sp}), 142, 144
Solubilization
  alternative equilibria for, 146 151
  and "enhanced solubility," 144 153
  ethanol on, 152 153
  hydrophilic polymers on, 152, 153
  of naproxen, 153
  pH with surfactants and, 151 152
  solid state, modifications to, 144 145
  solution phase, modifications to, 145
Solubilization techniques, 82 91
  cyclodextrins as solubilizers, 91
  for injectable formulations, 78
  pH adjustment, 82 83
  salt formation, 83 84
  surfactants as solubilizers, 87 91
  use of cosolvents, 85
Solutes
  hydrogen bonding and, 139
  lipophilicty of, 138 139
  macroscopic properties of, 140 141
  modifications in, 134, 146 151
  molar volume of, 140
  molecular connectivity in, 140
  molecular surface area of, 139 140
  polarizability of, 137 138
  topology and steric effect of, 139
Solutol HS 15, surfactant, 149
Solvates, solubility of, 140 141
Solvents
  alterations in, 134
  dielectric constants of, 79, 80, 136, 145
  for electrolytes, characteristics of, 81
  polarity of, 136
  solubility parameters of, 136
  surface tension of, 137
Spatial aggregation propensity, 198
Special purpose glasses, 288
Spike retention, 343
Spray drying technique
  for drug powder preparation, 107 108
  for microspheres, 174
Stability, 61 62
  actives, requirements for, 160
  cosolvent and, 85 87
  formulation development and, 77
[Stability]
  of protein drug, 72 73
  of suspensions, 163 164
Stability studies
  of protein products
    in use and compatibility, 244
    photostability, 243 244
    shelf life support, long term, 243
    shipping, 243
    temperature, 243
Stability testing, 376 377
Stabilizers, in protein formulations, 236 238
Staked needle, 330
Static light scattering (SLS) measures, 216 217
Steam sterilization, 350 351
Steric effect, solubility and, 139
"Sterile for suspension," 107
Sterile solids, 107
Sterility testing, 300, 376 377
Steroids, 264
Stokes' law, 163
Stones, 302
Stoppered vial flange, 359
Stress in glass containers, 302
Stroma, 257
Styrene butadiene rubber (SBR), 334
Subconjunctival injection, 20. *See also* Intraocular injection
Subcutaneous (SC) administration, 1, 12 13
  sites for, 13
Sublimation drying. *See* Primary drying
Subsaturation state, equilibrium and, 134, 135
Subtenon injection, 261 262
Sulfur dioxide (SO_2), 291
Supercooled liquid, 287
Supersaturation state, equilibrium and, 134, 135
Support studies in formulation development, 114 117
  biological considerations, 114 115
  manufacturing and handling studies, 115 117
Surface active drugs, 89 90
Surface chemistry, of a glass container, 300 301
  container finish dimensions and matching closures, 298
  mechanical and thermal characteristics of, 301 302
  quality attributes, 302 303
Surface tension, of solvent, 137
Surfactants, 315
  ionic, 88
  nonionic, 87 88
  in parenterals, 149
  selection for injectable products, 90
  solubility and, 60 61, 87 91
Suspensions
  content uniformity in delivery, 271
  formulation of
    drug particle size on, 164 165
    excipients on, 165 166
    flocculated system in, 163
    manufacture and control of, 166
    parenteral, 164 166

[Suspensions]
  and oily vehicle depot systems, 160 163
  parenteral, 70 71, 102 104
    methods for preparation of, 103 104
    requirements of, 103
  particle size distribution, 269 270
  physical stability of, 163 164, 270 271
Sustained release system. *See* Depot delivery systems
Sutures, resorbable
  polymers and, 167
SUV. *See* Small unilamellar vesicles (SUV)
Swelling, 339
SynchroMed pump, 182
Syringeability
  particle size on, 164
  of suspensions, 70
Syringe filling system, 242
Syringe pumps, 25 26. *See also* Parenteral drug delivery
  defined, 25
Syringes, prefilled. *See* Prefilled syringes
Systemic recirculation, 260

Tablet bottles, 310
Tandem mass spectrometry (MS/MS), 204 205
T cell dependent pathway, 45
T cell independent pathway, 45
Tear film, lipid layer of, 255
Tear fluid osmolality, 256
Technologies, enhancements of existing
  of depot delivery systems, 185 186
Teflon®, 319
Temperature
  collapse, 109
  effect on critical micelle concentration, 89
  effect on solubility, 81 82
  eutectic, 109 111
  on stability, 243
Terephthalic acid (TPA), 309
Terminal sterilization, 184, 291
  feasibility of, 116
Test chambers for flexible packages, 367
TGA. *See* Thermogravimetric analysis (TGA)
Thermal conductivity, of glass, 302
Thermal expansion coefficient, of glass container, 302
Thermal properties of, drug substance, 66 67
Thermal transition midpoint (T_m)
  in proteins, 224
Thermodynamic solubility, defined, 134
Thermogravimetric analysis (TGA), 66
Time of flight (TOF) mass analyzers, 202
Tip cap, 330
Tissue fluids, composition of, 119
Titanium oxide (Ti_2O_3), 291
TNF. *See* Tumor necrosis factor (TNF)
TOF. *See* Time of flight (TOF) mass analyzers
Tonicity
  calculation of, 100 101
[Tonicity]
  in formulation development, 100 102, 114, 120 121
  modifiers, in protein formulations, 238
Topas®, 306
Topical drugs administration, 258 260
Topological indices (TI), defined, 139
Total organic carbon (TOC) content, 307
Total parenteral nutrition (TPN) therapy, 122, 125
TPN. *See* Total parenteral nutrition (TPN) therapy
Trabecular meshwork, 257
Transitional flow, 363
Treated or nontreated containers, 291
Treated soda lime glass, 291
Truncated species, 214
Trypsin, for proteolytic mapping, 202
Tryptophan (Trp)
  fluorescence, 205, 208 210
  oxidation of, 230
Tumor necrosis factor (TNF), 45
Tungsten, 305
  pins, 316
Tuohy needle, 18
Turbulent flow, 362
Tyrosine (Tyr) fluorescence, 205, 208 210
Tyvek®, 360

UFDF. *See* Ultrafiltration and diafiltration (UFDF) step
Ultrafiltration and diafiltration (UFDF) step, 233
Ultrasonic welding, 360
Ultraviolet (UV) lamp
  CD spectroscopy and, 205 206
Ultraviolet wavelengths, absorption of, 289
U.S. compendia, 367
U.S. FDA Guidance for sterilization process, 376
U.S. Food and Drug Administration (FDA), 194
U.S. Pharmacopeia (USP)
  categorization of sterile preparations for parenteral use, 76
Use time stability studies, 116
USP. *See* U.S. Pharmacopeia (USP)
USP "powdered glass" test, 291
USP Surface Test, 291 292, 301
USP Water Attack at 121° Test, 291
UV. *See* Ultraviolet (UV) lamp

Vaccine formulations, in depot system, 163
Vacuum decay leak test, 367 370
Vacuum decay tool, 370
Validation, of elastomeric closure packaging, 353 354
Validation study, of stopper washing, 349 350
van der Waals forces, 136, 139, 163
Vapor sorption analysis, 67
Vascular endothelial growth factors (VEGF), 265
Vascular tunic
  blood retina barrier, 258
  choroid, 258

Vehicle
  for large volume parenterals, 122
  selection in, formulation development, 77, 78
  types of
    aqueous vehicle, 91
    nonaqueous vehicle, 91 92
Vello process, 293 294
Viadur, implant, 182
Vial breakage, 238
Vial package leakage, 380
Vials, 295 298, 305
  lyophilization and reconstitution, 312 314
  market considerations, 310 311
  process considerations, 314
  proteins and peptides, 311 312
  stoppers for, 327 329
  storage and transport at low temperatures, 312
Vial stopper seal systems, 300
Vitreous (glassy) silica, 289
Vitreous humor, 257
Vulcanization, 332

Washing of rubber closures, 325 326
Washing programs for elastomeric closures, 347 348
Water filled syringes, 315
Water for injection (WFI), 78, 91, 121, 122, 126
Water in oil in water ($w_1/o/w_2$) emulsion technique
  for microspheres, 173
Water interference, in formulations, 206
Water solubility, hydrotropes for, 147
Water vapor permeability, 338
"Wet" age related macular degeneration, 265
Wet media milling, 164
WFI. *See* Water for injection (WFI)
Wilco AG leak test systems, 369

X ray contrast media, 318
  packaging of, 318
X ray powder diffractometry (XRPD), 58
XRD. *See* Powder X ray diffraction (XRD)
XRPD. *See* X ray powder diffractometry (XRPD)

Zeonex®, 306
Zeonor®, 306
Zinc ion, 333
Zwitterions, solubility of, 142

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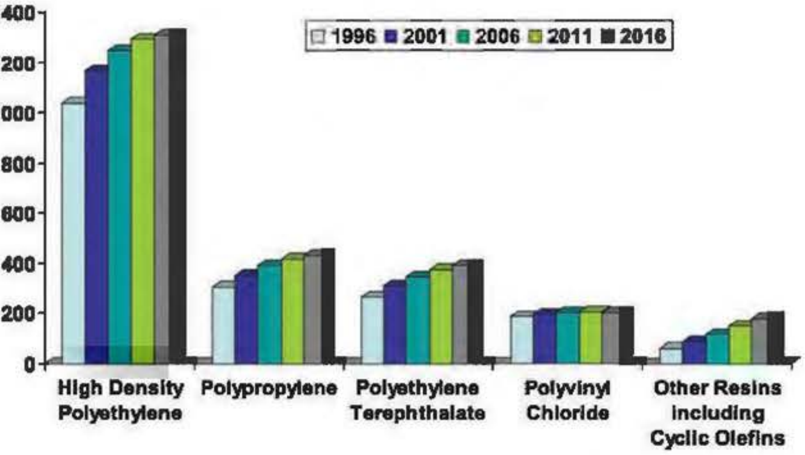

Figure 12.1 World pharmaceutical packaging plastics demand by resin (million pounds) (*see page 306*).

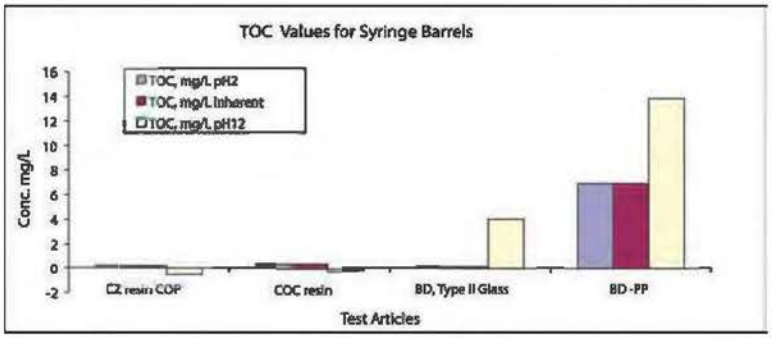

Figure 12.3 Comparison of total organic carbon as an extractable from syringe barrels. *Source*: Reproduced from Ref. 6 (*see page 307*).

Pharmaceutical Dosage Forms: Parenteral Medications
Third Edition
Volume 1: Formulation and Packaging

About the book

This three-volume set of *Pharmaceutical Dosage Forms: Parenteral Medications* is an authoritative, comprehensive reference work on the formulation and manufacture of parenteral dosage forms, effectively balancing theoretical considerations with the practical aspects of their development. As such, it is recommended for scientists and engineers in the pharmaceutical industry and academia, and will also serve as an excellent reference and training tool for regulatory scientists and quality assurance professionals.

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- A definitive chapter on container-closure integrity.
- New chapters on solubility and solubilization, formulation of depot delivery systems and biophysical/biochemical characterization of proteins.

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