Parenteral (Gk, para enteron, beside the intestine) dosage forms differ from all other drug dosage forms, because they are injected directly into body tissue through the primary protective systems of the human body, the skin, and mucous membranes. They must be exceptionally pure and free from physical, chemical, and biological contaminants. These requirements place a heavy responsibility on the pharmaceutical industry to practice current good manufacturing practices (cGMPs) in the manufacture of parenteral dosage forms and on pharmacists and other health care professionals to practice good aseptic practices (GAPs) in dispensing parenteral dosage forms for administration to patients.

Certain pharmaceutical agents, particularly peptides, proteins, and many chemotherapeutic agents, can only be given parenterally, because they are inactivated in the gastrointestinal tract when given by mouth. Parenterally-administered drugs are relatively unstable and generally highly potent drugs that require strict control of administration to the patient. Due to the advent of biotechnology, parenteral products have grown in number and usage around the world.

This chapter focuses on the unique characteristics of parenteral dosage forms and the basic principles for formulating, packaging, manufacturing, and controlling the quality of these unique products. The references and bibliography at the end of this chapter contain the most up-to-date texts, book chapters, and review papers on parenteral product formulation, manufacture, and quality control.
FORMULATION PRINCIPLES

Parenteral drugs are formulated as solutions, suspensions, emulsions, liposomes, microspheres, nanosystems, and powders to be reconstituted as solutions. This section describes the components commonly used in parenteral formulations, focusing on solutions and freeze-dried products. General guidance is provided on appropriate selection of the finished sterile dosage form and initial approaches used to develop the optimal parenteral formulation.

VEHICLES

WATER

Since most liquid injections are quite dilute, the component present in the highest proportion is the vehicle. The vehicle of greatest importance for parenteral products is water. Water of suitable quality for compounding and rinsing product contact surfaces, to meet United States Pharmacopoeia (USP) and other compendia specifications for Water for Injection (WFI), may be prepared either by distillation or by reverse osmosis. Only by these two methods is it possible to separate various liquid, gas, and solid contaminating substances from water. These two methods for preparation of WFI and specifications for WFI are also discussed in this chapter. With the possible exception of freeze-drying, there is no unit operation more important and none more costly to install and operate than that for the preparation of WFI.

WATER-MISCIBLE VEHICLES

A number of solvents that are miscible with water have been used as a portion of the vehicle in the formulation of parenterals. These solvents are used to solubilize certain drugs in an aqueous vehicle and to reduce hydrolysis. The most important solvents in this group are ethyl alcohol, liquid polyethylene glycol, and propylene glycol. Ethyl alcohol is used in the preparation of solutions of cardiac glycosides and the glycols in solutions of barbiturates, certain alkaloids, and certain antibiotics. Such preparations are given intramuscularly. There are limitations with the amount of these co-solvents that can be administered, due to toxicity concerns, greater potential for hemolysis, and potential for drug precipitation at the site of injection.2 Formulation scientists needing to use one or more of these solvents must consult the literature (e.g., Mottu F et al. 2000)2 and toxicologists to ascertain the maximum amount of co-solvents allowed for their particular product. Several references provide information on concentrations of co-solvents used in approved commercial parenteral products.3,4,5

NON-AQUEOUS VEHICLES

The most important group of non-aqueous vehicles is the fixed oils. The USP provides specifications for such vehicles, indicating that the fixed oils must be of vegetable origin so they will metabolize, will be liquid at room temperature, and will not become rancid readily. The USP also specifies limits for the free fatty acid content, iodine value, and saponification value (oil heated with alkali to produce soap, i.e., alcohol plus acid salt). The oils most commonly used are corn oil, cottonseed oil, peanut oil, and sesame oil. Fixed oils are used as vehicles for a number of solvents that are miscible with water have been used as a portion of the vehicle in the formulation of parenterals. These solvents include the following:

- Ethanol
- Propylene glycol

These solvents are used to solubilize certain drugs in an aqueous vehicle and to reduce hydrolysis. The most important solvents in this group are ethyl alcohol, liquid polyethylene glycol, and propylene glycol. Ethyl alcohol is used in the preparation of solutions of cardiac glycosides and the glycols in solutions of barbiturates, certain alkaloids, and certain antibiotics. Such preparations are given intramuscularly. There are limitations with the amount of these co-solvents that can be administered, due to toxicity concerns, greater potential for hemolysis, and potential for drug precipitation at the site of injection.2 Formulation scientists needing to use one or more of these solvents must consult the literature (e.g., Mottu F et al. 2000)2 and toxicologists to ascertain the maximum amount of co-solvents allowed for their particular product. Several references provide information on concentrations of co-solvents used in approved commercial parenteral products.3,4,5

SOLUTES

Care must be taken in selecting active pharmaceutical ingredients and excipients to ensure their quality is suitable for parenteral administration. A low microbial level will enhance the effectiveness of either the aseptic or the terminal sterilization process used for the drug product. Likewise, nonpyrogenic ingredients enhance the nonpyrogenicity of the finished injectable product. It is now a common GMP procedure to establish microbial and endotoxin limits on active pharmaceutical ingredients and most excipients. Chemical impurities should be virtually nonexistent in active pharmaceutical ingredients for parenterals, because impurities are not likely to be removed by the processing of the product. Depending on the chemical involved, even trace residues may be harmful to the patient or cause stability problems in the product. Therefore, manufacturers should use the best grade of chemicals obtainable and use its analytical profile to determine that each lot of chemical used in the formulation meets the required specifications.

Reputable chemical manufacturers accept the stringent quality requirements for parenteral products and, accordingly, apply good manufacturing practices to their chemical manufacturing. Examples of critical bulk manufacturing precautions include:

- Using dedicated equipment or properly validated cleaning to prevent cross-contamination and transfer of impurities;
- Using WFI for rinsing equipment;
- Using closed systems, wherever possible, for bulk manufacturing steps not followed by further purification; and
- Adhering to specified endotoxin and bioburden testing limits for the substance.

ADDED SUBSTANCES

The USP includes in this category all substances added to a preparation to improve or safeguard its quality. An added substance may:

- Increase and maintain drug solubility. Examples include complexing agents and surface active agents. The most commonly used complexing agents are the cyclodextrins, including Captisol. The most commonly used surface active agents are polyoxyethylene sorbitan monolaurate (Tween 20) and polyoxyethylene sorbitan monoleate (Tween 80);
- Provide patient comfort by reducing pain and tissue irritation, as do substances added to make a solution isotonic or near physiological pH. Common tonicity adjusters are sodium chloride, dextrose, and glycerin;
- Enhance the chemical stability of a solution, as do antioxidants, inert gases, chelating agents, and buffers;
- Enhance the chemical and physical stability of a freeze-dried product, as do cryoprotectants and lyoprotectants. Common protectants include sugars, such as sucrose and trehalose, and amino acids, such as glycine;
- Enhance the physical stability of proteins by minimizing self-aggregation or interfacial induced aggregation. Surface active agents serve nicely in this capacity;
- Minimize protein interaction with inert surfaces, such as glass and rubber and plastic. Competitive binders, such as albumin, and surface active agents are the best examples;
- Protect a preparation against the growth of microorganisms. The term ‘preservative’ is sometimes applied only to those substances that prevent the growth of microorganisms in a preparation. However, such limited use is inappropriate, being better used for all substances that act to retard or prevent the chemical, physical, or biological degradation of a preparation;
- Although not covered in this chapter, other reasons for adding solutes to parenteral formulations include sustaining and/or controlling drug release (polymers), maintaining the drug in a suspension dosage form (suspending agents, usually polymers and surface active agents), establishing emulsified dosage forms (emulsifying agents, usually amphiphilic polymers and surface active agents), and preparation of liposomes (hydrated phospholipids).
Although added substances may prevent a certain reaction from taking place, they may induce others. Not only may visible incompatibilities occur, but hydrolysis, complexation, oxidation, and other invisible reactions may decompose or otherwise inactivate the therapeutic agent or other added substances. Therefore, added substances must be selected with due consideration and investigation of their effect on the total formulation and the container-closure system.

ANTIMICROBIAL AGENTS

The USP states that antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to preparations contained in multiple-dose containers. The European Pharmacopeia requires multiple-dose products to be bacteriocidal and fungicidal. They must be present in adequate concentration at the time of use to prevent the multiplication of microorganisms inadvertently introduced into the preparation, while withdrawing a portion of the contents with a hypodermic needle and syringe. The USP provides a test for Antimicrobial Preservative Effectiveness to determine that an antimicrobial substance or combination adequately inhibits the growth of microorganisms in a parenteral product. Because antimicrobials may have inherent toxicity for the patient, the USP prescribes maximum concentrations of antimicrobial agents in single-dose containers. The USP also requires that products without preservatives be used immediately, although some package inserts define immediate use as within 3 hours after entering the primary package, or a longer usage period must be justified. Large-volume, single-dose containers may not contain an added antimicrobial preservative. Therefore, special care must be exercised in storing such products after the containers have been opened to prepare an admixture, particularly those that support the growth of microorganisms, such as total parenteral nutrition (TPN) solutions and emulsions. It should be noted that, although refrigeration slows the growth of most microorganisms, it does not prevent their growth.

Buffers are used to stabilize a solution against chemical degradation or, especially for proteins, physical degradation (i.e., aggregation and precipitation) which might occur if the pH changes appreciably. Buffer systems should have as low a buffering capacity as feasible, so as not to significantly disturb the body’s buffering systems when injected. In addition, the buffer type and concentration on the activity of the active ingredient must be carefully evaluated. Buffer components are known to catalyze degradation of drugs. The acid salts most frequently employed as buffers are citrates, acetates, and phosphates. Amino acid buffers, especially histidine, have become buffer systems of choice for controlling solution pH of monoclonal antibody solutions.

Antioxidants are frequently required to preserve products, due to the risk that many drugs, including proteins with methionine or cysteine amino acids conformationally exposed, are susceptible to oxidation. Sodium bisulfite and other sulfur-containing antioxidants are used most frequently. Ascorbic acid and its salts are also good antioxidants. The sodium salt of ethylenediaminetetraacetic acid (EDTA) has been found to enhance the activity of antioxidants, in some cases, by chelating metallic ions that would otherwise catalyze the oxidation reaction.

Displacing the air (oxygen) in and above the solution, by purging with an inert gas, such as nitrogen, can also be used as a means to control oxidation of a sensitive drug. Process control is required for assurance that every container is deaerated adequately and uniformly. However, conventional processes for removing oxygen from liquids and containers do not absolutely remove all oxygen. The only approach for completely removing oxygen is to employ isolator technology, where the entire atmosphere can be recirculating nitrogen or another non-oxygen gas.

Tonicity Agents are used in many parenteral and ophthalmic products to adjust the tonicity of the solution. Although it is the goal for every injectable product to be isotonic with physiologic fluids, this is not an essential requirement for small volume injectables administered intravenously. However, products administered by all other routes, especially into the eye or spinal fluid, must be isotonic. Injections into the subcutaneous tissue and muscles should also be isotonic to minimize pain and tissue irritation. The agents most commonly used are electrolytes and mono- or disaccharides.

Cryoprotectants and Lyoprotectants are additives that serve to protect biopharmaceuticals from adverse effects, due to freezing and/or drying of the product during freeze-dry processing. Sugars (non-reducing), such as sucrose or trehalose, amino acids, such as glycine or lysine, polymers, such as liquid polyethylene glycol or dextran, and polyols, such as mannitol or sorbitol, are all possible cryo- or lyoprotectants. Several theories exist to explain why these additives work to protect proteins against freezing and/or drying effects.11,12 Excipients that are preferentially excluded from the surface of the protein are the best cryoprotectants, and excipients that remain amorphous during and after freeze-drying serve best as lyoprotectants.

GENERAL GUIDANCE FOR DEVELOPING FORMULATIONS OF PARENTERAL DRUGS

The final formulation of a parenteral drug product depends on understanding the following factors that dictate the choice of formulation and dosage form.

1. Route of administration—Injections may be administered by such routes as intravenous, subcutaneous, intradermal, intramuscular, intraarticular, intraleisional, and intrathecal. The type of dosage form (solution,
3. Pharmacokinetics of the drug—Rates of absorption (for routes of administration other than intravenous or intra-articular), distribution, metabolism, and excretion for a drug have some effect on the selected route of administration and, accordingly, the type of formulation. For example, if the pharmacokinetic profile of a drug is very rapid, modified release dosage formulations may need developed. The dose of drug and the dosage regimen are affected by pharmacokinetics, so the size (i.e., concentration) of the dose will also influence the type of formulation and amounts of other ingredients in the formulation. If the dosage regimen requires frequent injections, then a multiple dose formulation must be developed, if feasible. If the drug is distributed quickly from the site injection, complexing agents or viscosity inducing agents may be added to the formulation to retard drug dissolution and transport.

4. Drug solubility—If the drug is insufficiently soluble in water at the required dosage, then the formulation must contain a co-solvent or a solute that sufficiently increases and maintains the drug in solution. If relatively simple formulation additives do not result in a solution, then a dispersed system dosage form must be developed. Solubility also dictates the concentration of drug in the dosage form.

5. Drug stability—if the drug has significant degradation problems in solution, then a freeze-dried or other sterile solid dosage form must be developed. Stability is sometimes affected by drug concentration that, in turn, might affect size and type of packaging system used. For example, if concentration must be low, due to stability and/or solubility limitations, then the size of primary container must be larger, and this might preclude the use of syringes, cartridges, and/or smaller vial sizes. Obviously, stability dictates the expiration date of the product that, in turn, determines the storage conditions. Storage conditions might dictate choice of container size, formulation components, and type of container. If a product must be refrigerated, then the container cannot be too large, and formulation components must be soluble and stable at colder conditions.

6. Compatibility of drug with potential formulation additives and packaging systems—it is well-known that drug-excipient incompatibilities frequently exist. Initial preformulation screening studies are essential to ensure that formulation additives, although possibly solving one problem, will not create another. Stabilizers, such as buffers and antioxidants, although chemically stabilizing the drug in one way, may also catalyze other chemical degradation reactions. Excipients and certain drugs can form insoluble complexes. Impurities in excipients can cause drug degradation reactions. Peroxide impurities in polymers may catalyze oxidative degradation reactions with drugs, including proteins, which are oxygen sensitive.

7. The use of silicone to lubricate vial rubber closures, syringe rubber plungers to coat the inner surface of glass syringes, and cartridges potentially can induce protein aggregation. Therefore, compatibility studies need to be designed to determine the potential for a new biopharmaceutical drug adversely affected by the presence of silicone applied to certain packaging surfaces. The increased popularity of laminated rubber closures and plungers has been due to the elimination of the need for applying silicone to these materials. Silicone coating is still required for glass syringes and cartridges, which provide new opportunities for the use of plastic syringes with biopharmaceuticals that further minimize the potential for incompatibilities between biopharmaceuticals and packaging systems.

8. Desired type of packaging—Selection of packaging (i.e., type, size, shape, color of rubber closure, label, and aluminum cap) is often based on marketing preferences and competition. Knowing the type of final package early in the development process aids the formulation scientist in being sure the product formulation will be compatible and elegant in that packaging system.

Table 26-1 provides steps involved in the formulation of a new parenteral drug product. This can also be viewed as a list of questions, of which the answers will facilitate decisions on the final formulation that should be developed.

<table>
<thead>
<tr>
<th>Table 26-1. Main Steps Involved in the Formulation of a New Parenteral Drug Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Obtain physical properties of active drug substance</td>
</tr>
<tr>
<td>a. Structure, molecular weight</td>
</tr>
<tr>
<td>b. “Practical” solubility in water at room temperature</td>
</tr>
<tr>
<td>c. Effect of pH on solubility</td>
</tr>
<tr>
<td>d. Solubility in certain other solvents</td>
</tr>
<tr>
<td>e. Unusual solubility properties</td>
</tr>
<tr>
<td>f. Isoelectric point for a protein or peptide</td>
</tr>
<tr>
<td>g. Hygroscopicity</td>
</tr>
<tr>
<td>h. Potential for water or other solvent loss</td>
</tr>
<tr>
<td>i. Aggregation potential for protein or peptide</td>
</tr>
<tr>
<td>2. Obtain chemical properties of active drug substance</td>
</tr>
<tr>
<td>a. Must have a “validatable” analytical method for potency and purity</td>
</tr>
<tr>
<td>b. Time for 10% degradation at room temperature in aqueous solution in the pH range of anticipated use</td>
</tr>
<tr>
<td>c. Time for 10% degradation at 5°C</td>
</tr>
<tr>
<td>d. pH stability profile</td>
</tr>
<tr>
<td>e. Sensitivity to oxygen</td>
</tr>
<tr>
<td>f. Sensitivity to light</td>
</tr>
<tr>
<td>g. Major routes of degradation and degradation products</td>
</tr>
<tr>
<td>3. Initial formulation approaches</td>
</tr>
<tr>
<td>a. Know timeline(s) for drug product</td>
</tr>
<tr>
<td>b. Know how drug product will be used in the clinic</td>
</tr>
<tr>
<td>i. Single dose vs multiple dose</td>
</tr>
<tr>
<td>ii. If multiple dose, will preservative agent be part of drug solution/powder or part of diluent?</td>
</tr>
<tr>
<td>iii. Shelf life goals</td>
</tr>
<tr>
<td>iv. Combination with other products, diluents</td>
</tr>
<tr>
<td>c. From knowledge of solubility and stability properties and information from anticipated clinical use, formulate drug with components and solution properties known to be successful at dealing with these issues, then perform accelerated stability studies.</td>
</tr>
</tbody>
</table>
significant manipulation of the product. For example, tablets and
manufacturer, are consumed by the patient without any sig-

Most dosage forms, when released to the marketplace by the
from a drug, it can usually be provided by the intravenous in-
select advantages. If immediate physiological action is needed

Injections may be classified in six general categories:

1. Solutions ready for injection
2. Dry, soluble products ready to be combined with a sol-
3. Suspensions ready for injection
4. Dry, insoluble products ready to be combined with a
vehicle just prior to use
5. Emulsions
6. Liquid concentrates ready for dilution prior to
administration

When compared with other dosage forms, injections possess
select advantages. If immediate physiological action is needed
from a drug, it can usually be provided by the intravenous in-
jection of an aqueous solution. Modification of the formulation
or another route of injection can be used to slow the onset and
prolong the action of the drug. The therapeutic response of a drug
is controlled more readily by parenteral administration, since
the irregularities of intestinal absorption are circumvented. Also,
since the drug is administered by a professionally trained per-
son, it confidently can be expected that the dose is accurately
administered. Drugs can be administered parenterally, when they
cannot be given orally, due to the unconscious or uncooperative
state of the patient or due to inactivation or lack of absorption
in the intestinal tract. Among the disadvantages of this dosage form
are the requirement of asepsis at administration, the risk of tissue
toxicity from local irritation, the real or psychological pain fac-
tor, and the difficulty in correcting an error, should one be made.
In the latter situation, unless a direct pharmacological antagonist
is immediately available, correction of an error may be impos-
able. One other disadvantage is that daily or frequent administra-
tion pose difficulties, as patients must either visit a professionally
trained person or learn to self-inject. However, the advent of
home health care as an alternative to extended institutional care
and availability of new medications from biotechnology to treat
chronic diseases has mandated the development of programs for
training lay persons to administer these dosage forms.

### ADMINISTRATION

Injections may be classified in six general categories:

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and availability of new medications from biotechnology to treat
chronic diseases has mandated the development of programs for
training lay persons to administer these dosage forms.

### PARENTERAL COMBINATIONS

Most dosage forms, when released to the marketplace by the
manufacturer, are consumed by the patient without any sig-
ificant manipulation of the product. For example, tablets and
capsules are ingested in the same form they were when released
by the manufacturer. For many parenteral drug products, this is
not the case. For example, products in vials must be withdrawn
into a syringe prior to injection and often combined with other
products in infusion solutions prior to administration. Freez-
edried products, first, have to be reconstituted with a specific
or non-specific diluent prior to being withdrawn from the vial.
Specifically, it is common practice for a physician to order the
addition of a small-volume therapeutic injection (SVI), such as
an antibiotic, to large-volume injections (LVIs), such as 1000
mL of 0.9% sodium chloride solution, to avoid the discomfort,
for the patient, of a separate injection. Certain aqueous vehicles
are recognized officially, due to their valid use in parenterals.
Often, they are used as isotonic vehicles to which a drug may
be added at the time of administration. The additional osmotic
effect of the drug may not be enough to produce any discomfort
when administered. These vehicles include Sodium Chloride
Injection, Ringer's Injection, Dextrose Injection, Dextrose and
Sodium Chloride Injection, and Lactated Ringer's Injection.

Although the pharmacist is the most qualified health profes-
sional to be responsible for preparing such combinations, as is
clearly stated in the hospital accreditation manual of the Joint
Commission on Accreditation of Healthcare Organizations,13
interactions among the combined products can be troublesome
for the pharmacist. In fact, incompatibilities can occur
and cause inactivation of one or more ingredients or other un-
desired reactions. Patient deaths have been reported from the
precipitate formed by two incompatible ingredients. In some
instances, incompatibilities are visible as precipitation or color
change, but, in other instances, there may be no visible effect.
The many potential combinations present a complex situa-
tion even for the pharmacist. To aid in making decisions con-
cerning potential problems, a valuable compilation of relevant
data has been assembled by Trissel14 and is updated regularly.
Further, the advent of computerized data storage and retrieval
systems has provided a means to organize and gain rapid access
to such information. (Further information on this subject may
be found in Chapter 27—Pharmaceutical Compounding – USP
<797> Sterile Preparations.)

As studies have been undertaken and more information has
been gained, it has been shown that knowledge of variable
factors, such as pH and the ionic character of the active con-
stituents, aids substantially in understanding and predicting po-
tential incompatibilities. Kinetic studies of reaction rates may
be used to describe or predict the extent of degradation. Ulti-
mately, a thorough study should be undertaken of each therapeu-
tic agent in combination with other drugs and IV fluids, not
only of generic, but also of commercial preparations, from the
physical, chemical, and therapeutic aspects.

Ideally, no parenteral combination should be administered,
unless it has been studied thoroughly to determine its effect on
the therapeutic value and safety of the combination. However,
such an ideal situation may not exist. Nevertheless, it is the re-
sponsibility of the pharmacist to be as familiar as possible with
the physical, chemical, and therapeutic aspects of parenteral
combinations and to exercise the best possible judgment as
whether or not the specific combination extemporaneously
prescribed is suitable for use in a patient.

### GENERAL CONSIDERATIONS

An inherent requirement for parenteral preparations is that
they be of the very best quality and provide the maximum safe-
ty for the patient. Constant adherence to high moral and pro-
fessional ethics on the part of the responsible persons is most
vital to achieving the desired quality in the products prepared.

### TYPES OF PROCESSES

The preparation of parenteral products may be categorized as
small-scale dispensing, usually one unit at a time, or large-scale
manufacturing in which hundreds of thousands of units may
constitute one lot of product. The former category illustrates the type of processing done in early clinical phase manufacturing or in institutions, such as hospital pharmacies. The latter category is typical of the processing done in the later clinical phase and commercial manufacturing in the pharmaceutical industry. Wherever they are made, parenteral products must be subjected to the same basic practices of GMPs and good aseptic processing essential for the preparation of a safe and effective sterile product of highest quality; however, the methods used must be modified appropriately for the scale of operation.

The small-scale preparation and dispensing of parenteral products might use sterile components in their preparation. Therefore, the overall process focuses on maintaining, rather than achieving, sterility in the process steps. In the hospital setting, the final product might have a shelf life measured in hours. However, the extensive movement of patients out of the hospital to home care has modified hospital dispensing of parenteral products, wherein multiple units are made for a given patient and a shelf life of 30 days or more is required. Such products are sometimes made in hospital pharmacies, but increasingly in centers set up to provide this service. A discussion of such processing can be found in the USP general chapter <1206>.

This chapter emphasizes the preparation of parenteral products from non-sterile components in the highly technologically advanced plants of the pharmaceutical industry, using cGMP principles. In the pursuit of cGMP, consideration should be given to:

1. Ensuring that the personnel responsible for assigned duties are capable and qualified to perform them.
2. Ensuring that ingredients used in compounding the product have the required identity, quality, and purity.
3. Validating critical processes to be sure the equipment used and the processes followed ensure that the finished product has the qualities expected.
4. Maintaining a production environment suitable for performing the critical processes required, addressing such matters as orderliness, cleanliness, asepsis, and avoidance of cross contamination.
5. Confirming, through adequate quality-control procedures, that the finished products have the required potency, purity, and quality.
6. Establishing, through appropriate stability evaluation, that the drug products retain their intended potency, purity, and quality, until the established expiration date.
7. Ensuring that processes are always carried out in accord with established, written procedures.
8. Providing adequate conditions and procedures for the prevention of mix-ups.
9. Establishing adequate procedures, with supporting documentation, for investigating and correcting failures or problems in production or quality control.
10. Providing adequate separation of quality-control responsibilities from those of production to ensure independent decision making.

The pursuit of cGMP is an ongoing effort that must flex with new technological developments and new understanding of existing principles. Due to the extreme importance of quality in health care of the public, US Congress has given the responsibility of regulatory scrutiny over the manufacture and distribution of drug products to the FDA. Therefore, the operations of the pharmaceutical industry are subject to the oversight of the FDA and, with respect to manufacturing practices, to the application of the cGMPs. (These regulations are discussed more fully in Chapter 3—Quality Assurance and Control.)

In concert with the pursuit of cGMPs, the pharmaceutical industry has shown initiative and innovation in the extensive technological development and improvement in quality, safety, and effectiveness of parenteral dosage forms in recent years. Examples include developments in:

- modular facility design and construction—smaller rooms, easier to clean, sanitize, and maintain;
- application of disposable technologies for compounding, mixing, and filling—reduce potential for cross-contamination;
- closure cleaning, siliconization (if applicable), and sterilization—all-in-one systems for rubber closures;
- sterilization technologies—well-defined sterilization validation principles, multiple approaches to sterilization cycles;
- filling technologies—greater speed, precision, and handling of viscous solutions;
- aseptic processing technology, including barrier isolator technology and restricted access barrier systems;
- freeze-drying technologies—automated loading and unloading, advances in process monitoring;
- control of particulate matter—greater diligence in cleaning methodologies, in-coming inspections, more experience with sources, causes, and minimization of particulate matter in facilities, on equipment and packaging, and personnel practices; and
- Automation—weight checking, inspection technologies, labeling and finishing operations.

### GENERAL MANUFACTURING PROCESS

The preparation of a parenteral product may encompass four general areas:

1. Procurement and accumulation of all components in a warehouse area, until released to manufacturing;
2. Processing the dosage form in appropriately designed and operated facilities;
3. Packaging and labeling in a quarantine area, to ensure integrity and completion of the product; and
4. Controlling the quality of the product throughout the process.

Procurement encompasses selecting and testing according to specifications of the raw-material ingredients and the containers and closures for the primary and secondary packages. Microbiological purity, in the form of bioburden and endotoxin levels, has become standard requirements for raw materials.

Processing includes cleaning containers and equipment to validated specifications, compounding the solution (or other dosage form), filtering the solution, sanitizing or sterilizing the containers and equipment, filling measured quantities of product into the sterile containers, stoppering (either completely or partially for products to be freeze-dried), freeze-drying, terminal sterilization (if possible), and final sealing of the final primary container.

Packaging normally consists of the labeling and cartoning of filled and sealed primary containers. Control of quality begins with the incoming supplies, being sure that specifications are met. Careful control of labels is vitally important, as errors in labeling can be dangerous for the consumer. Each step of the process involves checks and tests to ensure the required specifications at the respective step are being met. Labeling and final packaging operations are becoming more automated.

The quality control unit is responsible for reviewing the batch history and performing the release testing required to clear the product for shipment to users. A common FDA citation for potential violation of cGMP is the lack of oversight by the quality control unit in batch testing and review and approval of results.
Components of parenteral products include the active ingredient, formulation additives, vehicle(s), and primary container and closure. Establishing specifications to ensure the quality of each of these components of an injection is essential. Secondary packaging is relevant more to marketing considerations, although some drug products might rely on secondary packaging for stability considerations, such as added protection from light exposure for light-sensitive drugs and antimicrobial preservatives.

The most stringent chemical-purity requirements will normally be encountered with aqueous solutions, particularly if the product is sterilized at an elevated temperature where reaction rates will be accelerated greatly. Dry preparations pose relatively few reaction problems but may require definitive physical specifications for ingredients that must have certain solution or dispersion characteristics when a vehicle is added.

Containers and closures are in prolonged, intimate contact with the product and may release substances into, or remove ingredients from, the product. Rubber closures are especially problematic (sorption, leachables, air and moisture transmission properties), if not properly evaluated for compatibility with the final product. Assessment and selection of containers and closures are essential for final product formulation, to ensure the product retains its purity, potency, and quality during the intimate contact with the container throughout its shelf life. Administration devices (e.g., syringes, tubing, transfer sets) that come in contact with the product should be assessed and selected with the same care as are containers and closures, even though the contact period is usually brief.

**WATER FOR INJECTION (WFI)**

**PREPARATION**

The source water can be expected to be contaminated with natural suspended mineral and organic substances, dissolved mineral salts, colloidal material, viable bacteria, bacterial endotoxins, industrial or agricultural chemicals, and other particulate matter. The degree of contamination varies with the source and will be markedly different, whether obtained from a well or from surface sources, such as a stream or lake. Hence, the source water must be pretreated by one or a combination of the following treatments: chemical softening, filtration, deionization, carbon adsorption, or reverse osmosis purification. Figure 26-1 shows a schematic of a typical process used to convert potable water to Water for Injection.

Water for Injection can be prepared by distillation or by membrane technologies (i.e., reverse osmosis or ultrafiltration). The EP (European Pharmacopoeia) only permits distillation as the process for producing WFI. The USP and JP (Japanese Pharmacopoeia) allow all these technologies to be applied.

Distillation is a process of converting water from a liquid to its gaseous form (steam). Since steam is pure gaseous water, all other contaminants in the feedwater are removed. A conventional still consists of a boiler (evaporator), containing feed water (distilland); a source of heat to vaporize the water in the evaporator; a headspace above the level of distilland, with condensing surfaces for refluxing the vapor, thereby returning nonvolatile impurities to the distilland; and means for eliminating volatile impurities (demister/separation device) before the hot water vapor is condensed; and a condenser for removing the heat of vaporization, thereby converting the water vapor to a liquid distillate.

The specific construction features of a still and the process specifications have a marked effect on the quality of distillate obtained from a still. Several factors must be considered in selecting a still to produce WFI:

1. The quality of the feed water will affect the quality of the distillate. For example, chlorine in water, especially, can cause or exacerbate corrosion in distillation units, and silica causes scaling within. Controlling the quality of the feed water is essential for meeting the required specifications for the distillate.
2. The size of the evaporator will affect the efficiency. It should be large enough to provide a low vapor velocity, thus, reducing the entrainment of the distilland either as a film on vapor bubbles or as separate droplets.
3. The baffles (condensing surfaces) determine the effectiveness of refluxing. They should be designed for efficient removal of the entrainment at optimal vapor velocity, collecting and returning the heavier droplets contaminated with the distilland.
4. Redissolving volatile impurities in the distillate reduces its purity. Therefore, they should be separated efficiently from the hot water vapor and eliminated by aspirating them to the drain or venting them to the atmosphere.
5. Contamination of the vapor and distillate from the metal parts of the still can occur. Present standards for high-purity stills are that all parts contacted by the vapor or distillate should be constructed of metal coated with pure tin, 304 or 316 stainless-steel, or chemically resistant glass.

The design features of a still also influence its efficiency of operation, relative freedom from maintenance problems, or extent of automatic operation. Stills may be constructed of varying size, rated according to the volume of distillate that can be produced per hour of operation under optimum conditions. Only stills designed to produce high-purity water may be considered for use in the production of WFI. Conventional commercial stills designed for the production of high-purity water are available from several suppliers.

There are two basic types of WFI distillation units—the vapor compression still and the multiple effect still.

**Compression Distillation**

The vapor-compression still, primarily designed for the production of large volumes of high-purity distillate with low consumption of energy and water, is illustrated diagrammatically in Figure 26-2. To start, the feed water is heated from an external source in the evaporator to boiling. The vapor produced in the tubes is separated from the entrained distilland in the separator and conveyed to a compressor that compresses the vapor and raises its temperature to approximately 107°C. It then flows to the steam chest, where it condenses on the outer surfaces of the tubes containing the distilland; the vapor is, thus, condensed...
and drawn off as a distillate, while giving up its heat to bring the distilland in the tubes to the boiling point. Vapor-compression stills are available in capacities from 50 to 2800 gal/hr.

**Multiple-Effect Stills**

The multiple-effect still is also designed to conserve energy and water usage. In principle, it is simply a series of single-effect stills or columns running at differing pressures where phase changes of water take place. A series of up to seven effects may be used, with the first effect operated at the highest pressure and the last effect at atmospheric pressure. Figure 26-3 shows a schematic drawing of a multiple-effect still. Steam from an external source is used in the first effect to generate steam under pressure from feed water; it is used as the power source to drive the second effect. The steam used to drive the second effect condenses as it gives up its heat of vaporization and forms a distillate. This process continues until the last effect, when the steam is at atmospheric pressure and must be condensed in a heat exchanger. The capacity of a multiple-effect still can be increased by adding effects. The quantity of the distillate will also be affected by the inlet steam pressure; thus, a 600-gal/hr unit designed to operate at 115 psig steam pressure could be run at approximately 55 psig and would deliver about 400 gal/hr. These stills have no moving parts and operate quietly. They are available in capacities from about 50 to 7000 gal/hr.

**Reverse Osmosis (RO)**

As the name suggests, the natural process of selective permeation of molecules through a semipermeable membrane separating two aqueous solutions of different concentrations is reversed. Pressure, usually between 200 and 400 psig, is applied to overcome osmotic pressure and force pure water to permeate through the membrane. Membranes, usually composed of cellulose esters or polyamides, are selected to provide an efficient rejection of contaminant molecules in raw water. The molecules most difficult to remove are small inorganic molecules, such as sodium chloride. Passage through two membranes in series is sometimes used to increase the efficiency of removal of these small molecules and decrease the risk of structural failure of a membrane to remove other contaminants, such as bacteria and pyrogens.

Several WFI installations utilize both RO and distillation systems for generation of the highest quality water. Since feedwater to distillation units can be heavily contaminated and, thus, affect the operation of the still, water is first run through RO units to eliminate contaminants. (For additional information, see the book by Collentro.)

Whichever system is used for the preparation of WFI, validation is required to be sure that the system, consistently and reliably, produces the chemical, physical, and microbiological quality of water required. Such validation should start with the determined characteristics of the source water and include the pretreatment, production, storage, and distribution systems. All of these systems together, including their proper operation and maintenance, determine the ultimate quality of the WFI.

**Storage and Distribution**

The rate of production of WFI is not sufficient to meet processing demands; therefore, it is collected in a holding tank for subsequent use. In large operations, the holding tanks may have a capacity of several thousand gallons and be a part of a continuously operating system. In such instances, the USP requires that the WFI be held at a temperature too high for microbial growth, normally a constant 80°C.

The USP also permits the WFI to be stored at room temperature but for a maximum of 24 hours. Under such conditions,
the WFI is collected as a batch for a particular use with any unused water discarded within 24 hours. Such a system requires frequent sanitization to minimize the risk of viable microorganisms being present. The stainless-steel storage tanks in such systems are usually connected to a welded stainless-steel distribution loop, supplying the various use sites with a continuously circulating water supply. The tank is provided with a hydrophobic membrane vent filter capable of excluding bacteria and nonviable particulate matter. Such a vent filter is necessary to permit changes in pressure during filling and emptying. The construction material for the tank and connecting lines is usually electropolished 316L stainless steel with welded pipe. The tanks also may be lined with glass or a coating of pure tin. Such systems are very carefully designed and constructed and often constitute the most costly installation within the plant.

When the water cannot be used at 80°C, heat exchangers must be installed to reduce the temperature at the point of use. Bacterial retentive filters should not be installed in such systems, due to the risk of bacterial buildup on the filters and the consequent release of pyrogenic substances.

Figure 26-3. Multiple-effect water for injection distillation. Schematic (A) and (B) a space saving and energy efficient combination still and steam generator capable of delivering 750kg/hr (1650lb/hr) of pharmaceutical grade steam and 2200 liters/hr (580 gal/hr) of Water For Injection. (Courtesy of Getinge.)
Purity

Although certain purity requirements have been alluded to, the USP and EP monographs provide the official standards of purity for WFI and Sterile Water for Injection (SWFI).

The chemical and physical standards for WFI have changed in the past few years. The only physical/chemical tests remaining are the new total organic carbon (TOC), with a limit of 500 ppb (0.5 mg/L), and conductivity, with a limit of 1.3 μS/cm at 25°C or 1.1 μS/cm at 20°C. The former is an instrumental method capable of detecting all organic carbon present, and the latter is a three-tiered instrumental test measuring the conductivity contributed by ionized particles (in microSiemens or micromhos) relative to pH. Since conductivity is integrally related to pH, the pH requirement of 5 to 7 in previous revisions has been eliminated. The TOC and conductivity specifications are now considered adequate minimal predictors of the chemical/physical purity of WFI. However, the wet chemistry tests are still used when WFI is packaged for commercial distribution and for SWFI.

Biological requirements continue to be, for WFI, not more than 10 colony-forming units (CFUs)/100 mL and less than 0.25 USP endotoxin units/mL. The SWFI requirements differ in that, since it is a final product, it must pass the USP Sterility Test.

WFI and SWFI may not contain added substances. Bacteriostatic Water for Injection (BWFI) may contain one or more suitable antimicrobial agents in containers of 30 mL or less. This restriction is designed to prevent the administration of a large quantity of a bacteriostatic agent that would probably be toxic in the accumulated amount of a large volume of solution, even though the concentration was low.

The USP also provides monographs giving the specifications for Sterile Water for Inhalation and Sterile Water for Irrigation. The USP should be consulted for the minor differences between these specifications and those for SWFI.

CONTAINERS AND CLOSURES

Injectable formulations are packaged into containers made of glass or plastic. Container systems include ampoules, vials, syringes, cartridges, bottles, and bags (Fig. 26-4).

Ampoules are all glass, whereas bags are all plastic. The other containers can be composed of glass or plastic and must include rubber materials, such as rubber stoppers for vials and bottles and rubber plungers and rubber seals for syringes and cartridges. Irrigation solutions are packaged in glass bottles with aluminum screw caps.

Table 26-2 provides a generalized comparison of the three compatibility properties—leaching, permeation, and adsorption—of container materials most likely involved in the formulation of aqueous parenterals. Further, the integrity of the container/closure system depends on several characteristics, including container opening finish, closure modulus, durometer and compression set, and aluminum seal application force.

(Continent-closure integrity testing is discussed in the Quality Assurance and Control section.)

CONTAINER TYPES

GLASS

Glass is employed as the container material of choice for most SVIs. It is composed, principally, of silicon dioxide, with varying amounts of other oxides, such as sodium, potassium, calcium, magnesium, aluminum, boron, and iron. The basic structural network of glass is formed by the silicon oxide tetrahedron. Boric oxide will enter into this structure, but most of the other oxides do not. The latter are only loosely bound, are present in the network interstices, and are relatively free to migrate. These migratory oxides may be leached into a solution in contact with the glass, particularly during the increased reactivity of thermal sterilization. The oxides dissolved may hydrolyze to raise the pH of the solution and catalyze or enter into reactions. Additionally, some glass compounds will be attacked by solutions and, in time, dislodge glass flakes into the solution. Such occurrences can be minimized by the proper selection of the glass composition.

Types

The USP provides a classification of glass:

- Type I, a borosilicate glass;
- Type II, a soda-lime treated glass;
- Type III, a soda-lime glass; and
- NP, a soda-lime glass not suitable for containers for parenterals.

Type I glass is composed, principally, of silicon dioxide (~81%) and boric oxide (~13%), with low levels of the non-network-forming oxides, such as sodium and aluminum oxides. It is a chemically resistant glass (low leachability), also having a low thermal coefficient of expansion (CoE) (32.5 x 10⁻⁷ cm/cm-°C for 33 expansion glass; 51.0 x 10⁻⁷ cm/cm-°C for 51 expansion glass). In comparison, soda-lime glass has a thermal CoE of expansion of 8.36 x 10⁻⁷ cm/cm-°C. The lower the thermal CoE, the more dimensionally stable the glass against thermal expansion stress that can result in cracking.

Types II and III glass compounds are composed of relatively high proportions of sodium oxide (~14%) and calcium oxide (~8%). This makes the glass chemically less resistant. Both types melt at a lower temperature, are easier to mold into various shapes, and have a higher thermal coefficient of expansion. Although there is no one standard formulation for glass among manufacturers of these USP type categories, Type II glass has a lower concentration of the migratory oxides than Type III. In addition, Type II has been treated under controlled temperature and humidity conditions, with sulfur dioxide or other dealkalizers to neutralize the interior surface of the container. Although it remains intact, this surface increases substantially the chemical resistance of the glass. However, repeated exposures to sterilization and alkaline detergents break down this dealkalized surface and expose the underlying soda-lime compound.

The glass types are determined from the results of two USP tests: the Powdered Glass Test and the Water Attack Test. A
The following rules apply with respect to glass leachables:

- Relatively low levels of leachables at pH 4-8.
- Relatively high levels of leachables at pH > 9.
- Major leachables are silicon and sodium.
- Minor leachables include potassium, barium, calcium, and aluminum.
- Trace leachables include iron, magnesium, and zinc.
- Treated glass gives less extractables, if pH < 8.

**Delamination**—Delamination, or glass particulate formation, is caused by chemical attack on the glass matrix by the formulation solution, resulting in weakening of the glass and eventual dislodgement of flakes from the glass surface. These fragments can be subvisible in size and, thus, difficult to detect. Delamination is of particular concern in tubing vials, and susceptibility may be driven by many of the same heat history factors that influence alkali leaching potential (as measured by compendial methods as a pH shift), although measures to reduce alkali leaching (ammonium sulfate treatment) may be completely ineffective in reducing delamination susceptibility.

Certain components or characteristics of the pharmaceutical product formulation may enhance the potential for delamination of the product container. Formulations with elevated pH (particularly >8), high sodium chloride content, or containing specific buffer components, known to attack or solubilize components, of the glass matrix (phosphate, citrate, tartrate, EDTA) are of potential concern. In some cases, delamination may be reduced or eliminated by careful control of process parameters during the glass forming processes; lower heat levels during the conversion process may be critical. Ammonium sulfate-treated glass containers are also known to be more susceptible to delamination.

Although compendial chemical resistance testing may be predictive of delamination potential, in some cases, experience indicates that additional testing with the actual formulation or a surrogate is required to properly evaluate container selection. For example, a product that undergoes terminal sterilization
could be evaluated by multiple sterilization cycles in the formulation matrix, followed by filtration and microscopic examination of the filters for subvisible particles of delaminated glass. For sterile-fill applications, it is recommended that the glass containers be filled with formulation placebo (all components except the unstable API) at the pH release limit(s) for the product and be challenged with a single autoclave cycle or an accelerated aging study at 55°C for at least 4 weeks, followed by filtration and microscopic examination for glass particles.

Adsorption—Adsorption of drug to solution contact surfaces and consequent loss of potency of delivery solutions is a primary concern of container/solution compatibility and must be rigorously and formally evaluated during solution/container evaluation and stability studies. Glass containers are fairly inert surfaces, for most small drug products at relatively high concentrations, but pose a higher risk for therapeutic proteins and other smaller drug products formulated at low concentrations. Since adsorption is a surface phenomenon, increasing the surface area to volume ratio increases the risk of losses due to adsorption. Thus, small volume products carry higher risk for loss of potency due to adsorption and should be carefully evaluated for drug loss.16

Cracks and Scratches—Small cracks and scratches on glass containers can best be minimized by implementation of quality agreements between parenteral product manufacturers and container manufacturers. Not only does the glass container manufacturer need strict control procedures to minimize cracks and scratches from the time the container is formed until it reaches the finished product manufacturer, but there also needs to be high quality, 100% inspection practices by both glass and final product manufacturers. Also, local quality inspection procedures and practices need to have clearly understood definitions and a library of examples for what is defined as a crack and scratch. Cracks are considered unacceptable, whereas scratches are more of an esthetic indication of product elegance.

Type I glass will be suitable for all products, although sulfur dioxide treatment is sometimes used for even greater resistance to glass leachables. Because cost must be considered, one of the other, less-expensive types may be acceptable. Type II glass may be suitable, for example, for a solution that is buffered, has a pH below 7, or is not reactive with the glass. Type III glass is usually suitable for anhydrous liquids or dry substances. However, some manufacturer-to-manufacturer variation in glass composition should be anticipated within each glass type. Therefore, for highly chemically sensitive parenteral formulations, it may be necessary to specify both USP Type and specific manufacturer.

Schott developed a technology, called Plasma Impulse Chemical Vapor Deposition (PECVD), that coats the inner surface of Type I glass vials with an ultrathin film of silicon dioxide.13 This film forms a highly efficient diffusion barrier that practically eliminates glass leachables. Such treated glass is especially useful for drug products having high pH values, formulations with complexing agents, or products showing high sensitivity to pH shifts.

Physical Characteristics

Commercially available containers vary in size from 0.5 to 1000 mL. Sizes up to 100 mL may be obtained as ampoules and vials, and larger sizes as bottles. The latter are used for intravenous and irrigating solutions. Smaller sizes are also available as syringes and cartridges. Ampoules, syringes, and cartridges are drawn from glass tubing. The smaller vials may be made by molding or from tubing. Larger vials and bottles are made only by molding. Containers made by drawing tubing are optically cleaner and have a thinner wall than molded containers (Fig. 26-4). Compared to molded glass, tubing glass also has better wall and finish dimensional consistency and no seams, is easier to label, weighs less, facilitates inspection, and has lower tooling costs. Tubing glass is preferable to molded glass for freeze-dried products, due to more efficient heat transfer from the shelf into the product. Molded containers are uniform in external dimensions, stronger, and heavier. Also, molded glass is not as susceptible to leachables and delamination, because the glass formation temperatures to vaporize and condense the alkali components of the glass are not as high as for tubing container manufacture.20 Easy-opening ampoules that permit the user to break off the tip at the neck constriction, without the use of a file, are weakened at the neck, by scoring or applying a ceramic paint with a different coefficient of thermal expansion. An example of a modification of container design to meet a particular need is the double-chambered vial, designed to contain a freeze-dried product in the lower, and solvent in the upper chamber. Other examples are wide-mouth ampoules with flat or rounded bottoms to facilitate filling with dry materials or suspensions and various modifications of the cartridge for use with disposable dosage units.

Glass containers must be strong enough to withstand the physical shocks of handling and shipping and the pressure differentials that develop, particularly during the autoclave sterilization cycle. They must be able to withstand the thermal shock resulting from large temperature changes during processing, for example, when the hot bottle and contents are exposed to room air at the end of the sterilization cycle. Therefore, a glass with a low coefficient of thermal expansion is necessary. The container must also be transparent to permit inspection of the drug.

Preparations that are light-sensitive must be protected, by placing them in amber glass containers or by enclosing flint glass containers in opaque cartons labeled to remain on the container during the period of use. It should be noted that the amber color of the glass is imparted by the incorporation of potentially leachable heavy metals, mostly iron and manganese, which may act as catalysts for oxidative degradation reactions. Silicone coatings are sometimes applied to containers to produce a hydrophobic surface, for example, as a means of reducing the friction of a rubber-tip of a syringe plunger.

The size of single-dose containers is limited to 1000 mL by the USP, and multiple-dose containers to 30 mL, unless stated otherwise in a particular monograph. Multiple-dose vials are limited in size to reduce the number of punctures for withdrawing doses and the accompanying risk of contamination of the contents. As the name implies, single-dose containers are opened or penetrated with aseptic care, and the contents used at one time. These may range in size from 1000-mL bottles to 1-mL or less ampoules, vials, or syringes. The integrity of the container is destroyed when opened, so that the container cannot be closed and reused.

A multiple-dose container is designed so that more than one dose can be withdrawn at different times, the container maintaining a seal between uses. It should be evident that, with full aseptic precautions, including sterile syringe and needle for withdrawing the dose and disinfection of the exposed surface of the closure, there is still a substantial risk of introducing contaminating micro-organisms and viruses into the contents of the vial. Due to this risk, the USP requires that all multiple-dose vials contain an antimicrobial agent or be inherently antimicrobial, as determined by the USP Antimicrobial Preservatives-Effectiveness tests. There are no comparable antiviral effectiveness tests, nor are antiviral agents available for such use. In spite of the advantageous flexibility of dosage provided by multiple-dose vials, single-dose, disposable container units provide the clear advantage of greater sterility assurance and patient safety.

Due to concerns for user safety and glass particulate matter occurring when glass is broken, glass sealed ampoules are no longer glass containers of choice for new SVIs in the United States.

RUBBER CLOSURES

To permit introduction of a needle from a hypodermic syringe into a multiple-dose vial and provide for rescaling as soon as the needle is withdrawn, each vial is sealed with a rubber
closure held in place by an aluminum cap (Fig. 26-5). This principle is also followed for single-dose containers of the cartridge type, except that there is only a single introduction of the needle to make possible the withdrawal or expulsion of the contents.

Rubber closures are composed of multiple ingredients plasticized and mixed together at an elevated temperature on milling machines. The elastomer primarily used in rubber closures, plungers, and other rubber items used in parenteral packaging and delivery systems is synthetic butyl or halobutyl rubber. Natural rubber is also used, but, if it is natural rubber latex, then the product label must include a warning statement, due to the potential for allergic reactions from latex exposure.

The plasticized mixture is placed in molds and vulcanized (cured) under high temperature and pressure. During vulcanization the polymer strands are cross-linked by the vulcanizing agent, assisted by the accelerator and activator, so that motion is restricted and the molded closure acquires the elastic, resilient character required for its use. Ingredients not involved in the cross-linking reactions remain dispersed within the compound and, along with the degree of curing, affect the properties of the finished closure. Table 26-3 provides examples of rubber-closure ingredients.

The physical properties considered in the selection of a particular formulation include elasticity, hardness, tendency to fragment, and permeability to vapor transfer. The elasticity is critical in establishing a seal with the lip and neck of a vial or other opening and in resealing after withdrawal of a hypodermic needle from a vial closure. The hardness should provide firmness, but not excessive resistance to the insertion of a needle through the closure, and minimal fragmentation of pieces of rubber should occur as the hollow shaft of the needle is pushed through the closure. Although vapor transfer occurs to some degree with all rubber formulations, appropriate selection of ingredients makes it possible to control the degree of permeability. Physicochemical and toxicological tests for evaluating rubber closures are described in section <381> in the USP.

The ingredients dispersed throughout the rubber compound may be subject to leaching into the product contacting the closure. These ingredients (Table 26-3) pose potential compatibility interactions with product ingredients, if leached into the product solution, and these effects must be evaluated. Further, some ingredients must be evaluated for potential toxicity.

The example of pure red cell aplasia, an immunogenic reaction caused by leachables from a rubber closure in a erythropoietin prefilled syringe formulation, highlights the criticality of appropriate container-closure and the study of such leachables and extractables, even as a function of stability shelf life.21

To reduce the problem of leachables, laminates have been applied to the product contact surfaces of closures, with various polymers, the most successful being Telflon® (DuPont polytetrafluoroethylene [PTFE]) and Fluorotec® (West/Daiikyo copolymer of tetrafluoroethylene and ethylene). Polymeric coatings have been developed that are claimed to have more integral binding with the rubber matrix, however, details of their function are trade secrets. Although rubber coatings do reduce the potential for extractables/leachables and eliminate the need for applied silicone treatment, they may have potential disadvantages of not flowing as easily during high speed filling operations and may not have the same container-closure integrity as uncoated stoppers with vial openings.

The physical shape of some typical closures may be seen in Figure 26-5. Most of them have a lip and a protruding flange that extends into the neck of the vial or bottle. Many disk closures

| **Table 26-3. Examples of Ingredients Found in Rubber Closures** |
|-------------------|-------------------|
| **Ingredient**    | **Examples**      |
| Elastomer         | Natural rubber (latex) |
|                   | Butyl rubber      |
|                   | Neoprene          |
| Vulcanizing (curing agent) | Sulfur |
|                   | Peroxides         |
| Accelerator       | Zinc dibutylthiophosphoramide |
| Activator         | Zinc oxide        |
|                   | Stearic acid      |
| Antioxidant       | Dilauryl thiodipropionate |
| Plasticizer/lubricant | Paraffinic oil   |
|                   | Silicone oil      |
| Fillers           | Carbon black      |
|                   | Clay              |
|                   | Barium sulfate    |
| Pigments          | Inorganic oxides  |
|                   | Carbon black      |
are being used now, particularly in the high-speed packaging of antibiotics. Slotted closures are used on freeze-dried products to permit the escape of water vapor, since they are inserted only partway into the neck of the vial until completion of the drying phase of the cycle. Also, the top design of the freeze-dry closure is important to minimize sticking of the closure to underneath the dryer shelf after stopping the vial. Stoppers normally have a small protruding circle at the center of the top of the stopper. Gaps provided within the protruding circle minimize the tendency of the stopper to stick to the freeze-dryer shelf.

The plunger type of rubber is used to seal one end of a syringe or cartridge. At the time of use, the plunger expels the product by a needle inserted through the closure at the distal end of the package. Intravenous solution closures often have permanent holes for adapters of administration sets; irrigating solution closures are usually designed for pouring.

Rubber closures must be ‘slippery’ to move easily through a rubber closure hopper and other stainless steel passages, until they are fitted onto the filled vials. Traditionally, rubber materials are ‘siliconized’ (silicone oil or emulsion applied onto the rubber) to produce such lubrication. However, advances in rubber closure technologies have introduced closures that do not require siliconization, due to a special polymer coating applied to the outer surface of the closure. Examples are the Daikyo/West closures (Flurotec) and the Helvoet (Omniflex) closures.

PLASTIC

Thermoplastic polymers have been established as packaging materials for sterile preparations, such as large-volume parenterals, ophthalmic solutions, and, increasingly, small-volume parenterals. For such use to be acceptable, a thorough understanding of the characteristics, potential problems, and advantages for use must be developed. Three principal problem areas exist in using these materials:

1. Permeation of vapors and other molecules in either direction through the wall of the plastic container;
2. Leaching of constituents from the plastic into the product; and
3. Sorption (absorption and/or adsorption) of drug molecules or ions on the plastic material.

Permeation, the most extensive problem, may be troublesome by permitting volatile constituents, water, or specific drug molecules to migrate through the wall of the container to the outside and, thereby, be lost. This problem has been resolved, for example, by the use of an overwrap in the packaging of IV solutions in PVC bags to prevent loss of water during storage. Reverse permeation in which oxygen or other molecules may penetrate to the inside of the container and cause oxidative or other degradation of susceptible constituents may also occur. Leaching may be a problem, when certain constituents in the plastic formulation, such as plasticizers or antioxidants, migrate into the product. Thus, plastic polymer formulations should have as few additives as possible, an objective characteristically achievable for most plastics used for parenteral packaging. Sorption is a problem on a selective basis, that is, sorption of a few drug molecules occurs on specific polymers. For example, sorption of insulin and other proteins, vitamin A acetate, and warfarin sodium has been shown to occur on PVC bags and tubing, when these drugs were present as additives in IV admixtures. Table 26–2 gives a brief summary of some of these compatibility relationships.

One of the principle advantages of using plastic packaging materials is that they are not breakable, as is glass; also, there is a substantial weight reduction. The flexible bags of polyvinyl chloride or select polyolefins, currently in use for large-volume intravenous fluids, have the added advantage that no air inter-change is required; the flexible wall simply collapses as the solution flows out of the bag.

Most plastic materials have the disadvantage of not being as clear as glass, and, therefore, inspection of the contents is impeded. However, recent technologies have overcome this limitation, evidenced by plastic resins, such as CZ (polycyclopentane, Daikyo Seiko) and Topas COC (cyclic olefin copolymer, Tecona). In addition, many of these materials soften or melt under the conditions of thermal sterilization. However, careful selection of the plastic used and control of the autoclave cycle has made thermal sterilization of some products possible, large-volume injectables, in particular. Ethylene oxide or radiation sterilization may be employed for the empty container with subsequent aseptic filling. However, careful evaluation of the residues from ethylene oxide or its degradation products and their potential toxic effect must be undertaken. Investigation is required concerning potential interactions and other problems that may be encountered when a parenteral product is packaged in plastic.

Future trends in primary packaging for parenterals will continue to see significant growth in the application of plastic vials and syringes and the manufacturing of such packaging by form- (or blow-) fill-seal technologies. (For further details, see Chapter 35 (Pharmaceutical Packaging) and the review book chapter by Viltalam and DeGrazio.)

**NEEDLES**

Historically, stainless steel needles have been used to penetrate the skin and introduce a parenteral product inside the body. The advent of needleless injection systems has obviated the need for needles for some injections (e.g., vaccines) and is gaining in popularity over the conventional syringe and needle system. However, needleless injections are more expensive, can still produce pain on injection, are, potentially, a greater source of contamination (and cross-contamination from incessant use), and may not be as efficient in dose delivery.

Needles are hollow devices composed of stainless steel or plastic. Needles are available in a wide variety of lengths, sizes, and shapes. Needle lengths range from ¼ inch to 6 inches. Needle size is referred to as its gauge (G), or the outside diameter (OD) of the needle shaft. Gauge ranges are 11 to 32 G, with the largest gauge for injection usually being no greater than 16 G. 16 G needles have an OD of 0.065 inches (1.65 mm), whereas 32 G have an OD of 0.009 inches (0.20 mm). Needle shape includes regular, short bevel, intradermal, and winged. Needle shape is defined by one end of a needle enlarged to form a hub with a delivery device, such as a syringe, or other administration device. The other end of the needle is beveled, meaning it forms a sharp tip to maximize ease of insertion.

The route of administration, type of therapy, and whether the patient is a child or adult dictate the length and size of needle used. Intravenous injections use 1–2 inch 15–25 G needles. Intramuscular injections use 1–2 inch 19–22 G needles. Subcutaneous injections use ½–5/8 inch 24–25 G needles. Needle gauge for children rarely is larger than 22 G, usually 25–27G. Winged needles are used for intermittent heparin therapy. Many different types of therapies (e.g., radiology, anesthesia, biopsy, cardiovascular, ophthalmic, transfusions, tracheotomy, etc.) have their own peculiar types of needle preferences.

Needles are purchased either alone (e.g., Luer-Lok) to be attached to syringes, cartridge, and other delivery systems, or, for syringes, can be part of the syringe set (stake needle). Syringes with needles may also have needle protectors (for example, see http://www.bd.com/vacutainer/ pdfs/blood_transfer_device_ with_safetyglide_needle_VS5985.pdf) to avoid potential dangers of accidental needle stick post-administration (for more detail regarding the 2000 Needlestick Safety Act, see http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=106_cong_public_laws&docid=f:publ106.106). Such protectors can either be part of the assembly or be assembled during the finishing process. Needlestick prevention can be manual (shield activated manually by the user, although there is still the risk of accidental sticking), active (automated needle shielding activated by user), or passive (automated needle shielding without action by the user).
PYROGENS (ENDOTOXINS)

Water and packaging materials are the greatest sources of pyrogens (pyrogenic contamination). Pyrogens are products of metabolism of microorganisms. The most potent pyrogenic substances (endotoxins) are constituents (lipopolysaccharides, LPS) of the cell wall of gram-negative bacteria (e.g., Pseudomonas sp, Salmonella sp, Escherichia coli). Gram-positive bacteria and fungi also produce pyrogens but of lower potency and of different chemical nature. Gram positive bacteria produce peptidoglycans, whereas fungi produce β-glucans, both of which can cause non-endotoxin pyrogenic responses. Endotoxins are lipopolysaccharides that exist in high molecular weight aggregate forms. However, the monomer unit of LPS is less than 10,000 daltons, enabling endotoxin to easily pass through sterilizing 0.2 micron filters. The lipid portion of the molecule is responsible for the biological activity. Since endotoxins are the most potent pyrogens and gram-negative bacteria are ubiquitous in the environment, especially water, this discussion focuses on endotoxins and the risk of their presence as contaminants in sterile products.

Pyrogens, when present in parenteral drug products and injected into patients, can cause fever, chills, pain in the back and legs, and malaise. Although pyrogenic reactions are rarely fatal, they can cause serious discomfort and, in the seriously ill patient, shock-like symptoms that can be fatal. The intensity of the pyrogenic response and its degree of hazard are affected by the medical condition of the patient, the potency of the pyrogen, the amount of the pyrogen, and the route of administration (intrathecal is most hazardous followed by intravenous, intramuscular, and subcutaneous). When bacterial (exogenous) pyrogens are introduced into the body, LPS targets circulating mononuclear cells (monocytes and macrophages) that, in turn, produce pro-inflammatory cytokines, such as interleukin 2, interleukin 6, and tissue necrosis factor. Besides LPS, gram-negative bacteria also release many peptides (e.g., exotoxin A, peptidoglycan, and muramyl peptides) that can mimic the activity of LPS and induce cytokine release. The Limulus Amoebocyte Lysate (LAL) test can only detect the presence of LPS. It has been suggested that the Monocyte Activation Test, replace LAL as the official pyrogen test, due to its greater sensitivity to all agents that induce the release of cytokines that cause fever and a potential cascade of other adverse physiological effects.

CONTROL OF PYROGENS

It is impractical, if not impossible, to remove pyrogens, once present, without adversely affecting the drug product. Therefore, the emphasis should be on preventing the introduction or development of pyrogens in all aspects of the compounding and processing of the product.

Pyrogens may enter a preparation through any means that will introduce living or dead micro-organisms. However, current technology permits the control of such contamination, and the presence of pyrogens in a finished product indicates processing under inadequately controlled conditions. It also should be noted that time for microbial growth to occur increases the risk for elevated levels of pyrogens. Therefore, compounding and manufacturing processes should be carried out as expeditiously as possible, preferably planning completion of the process, including sterilization, within the maximum allowed time, according to process validation studies. Aseptic processing guidelines require establishment of time limitations throughout processing for the primary purpose of preventing the increase of endotoxin (and microbial) contamination that, subsequently, cannot be destroyed or removed.

Pyrogens can be destroyed by heating at high temperatures. A typical procedure for depyrogenation of glassware and equipment is maintaining a dry heat temperature of 250°C for 45 min. Exposure of 650°C for 1 min or 180°C for 4 hours, likewise, will destroy pyrogens. The usual autoclaving cycle will not do so. Heating with strong alkali or oxidizing solutions destroys pyrogens. It has been claimed that thorough washing with detergents will render glassware pyrogen-free, if subsequently rinsed thoroughly with pyrogen-free water. Rubber stoppers cannot withstand pyrogen-destructive temperatures, so reliance must be on an effective sequence of washing, thorough rinsing with WFI, prompt sterilization, and protective storage to ensure adequate pyrogen control. Similarly, plastic containers and devices must be protected from pyrogenic contamination during manufacture and storage, since known ways of destroying pyrogens affect the plastic adversely. It has been reported that anion-exchange resins and positively-charged membrane filters remove pyrogens from water. Also, although reverse osmosis membranes will eliminate them, the most reliable method for their elimination from water is distillation.

A method that has been used for the removal of pyrogens from solutions is adsorption on adsorptive agents. However, since the adsorption phenomenon may also cause selective removal of chemical substances from the solution, this method has limited application. Other in-process methods for their destruction or elimination include selective extraction procedures and careful heating with dilute alkali, dilute acid, or mild oxidizing agents. In each instance, the method must be studied thoroughly to be sure it will not have an adverse effect on the constituents of the product. Although ultrafiltration now makes pyrogen separation on a molecular-weight basis possible and the process of tangential flow is making large-scale processing more practical, use of this technology is limited, except in biotechnological processing.

SOURCES OF PYROGENS

Through understanding the means by which pyrogens may contaminate parenteral products, their control becomes more achievable. Therefore, it is important to know that water is probably the greatest potential source of pyrogenic contamination, since water is essential for the growth of micro-organisms and frequently contaminated with gram-negative organisms. When micro-organisms metabolize, pyrogens will be produced. Therefore, raw water can be expected to be pyrogenic and only when it is appropriately treated to render it free from pyrogens, such as WFI, should it be used for compounding the product or rinsing product contact surfaces, such as tubing, mixing vessels, and rubber closures. Even when such rinsed equipment and supplies are left wet and improperly exposed to the environment, there is a high risk they will become pyrogenic. Although proper distillation will provide pyrogen-free water, storage conditions must be such that micro-organisms are not introduced and subsequent growth is prevented.

Other potential sources of contamination are containers and equipment. Pyrogenic materials adhere strongly to glass and other surfaces, especially rubber closures. Residues of solutions in used equipment often become bacterial cultures, with subsequent pyrogenic contamination. Since drying does not destroy pyrogens, they may remain in equipment for long periods. Adequate washing reduces contamination, and subsequent dry-heat treatment can render contaminated equipment suitable for use. However, all such processes must be validated to ensure effectiveness. Aseptic processing guidelines require validation of the depyrogenation process by demonstrating at least 3-log reduction in an applied endotoxin challenge.

Solutions may be a source of pyrogens. For example, the manufacturing of bulk chemicals may involve the use of pyrogenic water for process steps, such as crystallization, precipitation, or washing. Bulk drug substances derived from cell culture fermentation will almost certainly be heavily pyrogenic. Therefore, all lots of solutes used to prepare parenteral products should be tested to ensure they will not contribute unacceptable quantities of endotoxin to the finished product. It is standard practice, today, to establish valid endotoxin limits on active pharmaceutical ingredients and most solute additives.
The manufacturing process must be carried out with great care and as rapidly as possible, to minimize the risk of microbial contamination. Preferably, no more product should be prepared than can be processed completely within one working day, including sterilization.

**PRODUCTION FACILITIES**

The production facility and its associated equipment must be designed, constructed, and operated properly for the manufacture of a sterile product to be achieved at the quality level required for safety and effectiveness. Materials of construction for sterile product production facilities must be ‘smooth, cleanable, and impervious to moisture and other damage’. Further, the processes used must meet cGMP standards. Since the majority of SVIs are aseptically processed (finished product not terminally sterilized), strict adherence to cGMP standards with respect to sterility assurance (particularly, the FDA and EU aseptic processing guidance documents, which can be found at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070342.pdf and Eudraex Vol 4, Annex I. Manufacture of Sterile Medicinal Products, http://ec.europa.eu/health/files/eudralex/vol-4/2008_11_25_gmp-ani1_en.pdf) is essential.

**FUNCTIONAL AREAS**

To achieve the goal of a manufactured sterile product of exceptionally high quality, many functional production areas are involved: warehousing or procurement; compounding (formulation); materials (containers, closures, equipment) preparation; filtration and sterile receiving; aseptic filling; stoppering; lyophilization (if warranted); and packaging, labeling, and quarantine. The extra requirements for the aseptic area are designed to provide an environment where a sterile fluid may be exposed to the environment for a brief period during subdivision from a bulk container to individual-dose containers, without becoming contaminated. Contaminants, such as dust, lint, and other particles and micro-organisms, are found floating in the air, lying on counters and other surfaces, attached to clothing and body surfaces of personnel, concentrated in the exhaled breath of personnel, and deposited on the floor. The design and control of an aseptic area is directed toward reducing the presence of these contaminants, so they are no longer a hazard to aseptic filling.

Although the aseptic area must be adjacent to support areas, so an efficient flow of components may be achieved, barriers must be provided to minimize ingress of contaminants to the critical aseptic area. Such barriers may consist of a variety of forms, including sealed walls, manual or automatic doors, air-lock pass-throughs, ports of various types, or plastic curtains. Figure 26-6 shows an example of a floor plan for a clinical supply production facility (selected as an example of a small-scale, noncomplex facility) in which the two fill rooms and the staging area constitute the walled critical aseptic area, access to which is only by means of pass-through airlocks. Adjacent support areas (rooms) consist of glass preparation, equipment wash, capping, manufacturing (compounding), and various storage areas. Figure 26-7 shows an example of a Class 100/Grade A small scale filling room with operators properly gowned and practicing good aseptic techniques.

**FLOW PLAN**

In general, the components for a parenteral product flow from the warehouse, after release, to either the compounding area, as for ingredients of the formula, or the materials support area, as for containers and equipment. After proper processing in these areas, the components flow into the security of the aseptic area for filling of the product in appropriate containers. From there the product passes into the quarantine and packaging area, where it is held until all necessary tests have been performed. If the product is to be sterilized in its final container, its passage is interrupted after leaving the aseptic area for subjecting to the sterilization process. After the results from all tests are known, the batch records have been reviewed, and the product has been found to comply with its release specifications, it passes to the finishing area for final release for shipment. There, sometimes, are variations from this flow plan to meet the specific needs of an individual product or to conform to existing facilities. Automated operations have much larger capacity and convey the components from one area to another with little or no handling by operators.

**Clean Room Classified Areas**

Due to the extremely high standards of cleanliness and purity that must be met by parenteral products, it has become standard practice to prescribe specifications for the environments (clean rooms) in which these products are manufactured (Table 26-4). Table 26-4A compares US and European classifications and
clean room designations assigned by the International Society of Pharmaceutical Engineers. Table 26-4B provides the International Standards Organization (ISO) 14644 Classification of Cleanroom Particle Limits adhered to by the parenteral manufacturing industry. Table 26-4A numbers are based on the maximum allowed number of airborne particles/l ft³ or particles/m³ of 0.5 μm or larger size and, for Europe, 5.0 μm or larger size. The classifications used in pharmaceutical practice normally range from Class 100,000 (Grade D) for materials support areas to Class 100 (Grade A) for aseptic areas. To achieve Class 100 conditions, HEPA filters are required for the incoming air, with the effluent air sweeping the downstream environment at a uniform velocity, 100 ft/min ± 20%, along parallel lines (laminar airflow). HEPA filters are defined as 99.99% or more efficient in removing, from the air, 0.3 μm particles generated by vaporization of the hydrocarbon Emory 3004.

Because so many parenteral products are manufactured at one site for global distribution, air quality standards in aseptic processing areas must meet both US and European requirements. European standards differ from US standards, as European standards:

- use Grades A, B, C, and D classifications, rather than Class X (100, 1,000, etc);
- use particle and microbial limits per cubic meter, rather than per cubic foot;
- require particle measurements at 5 microns in addition to 0.5 microns in Grade A and B areas; and
- differentiate area cleanliness dynamically and ‘at rest.’

For the sake of convenience, the remainder of this chapter uses Class X (e.g., 100, 1,000, 10,000, 100,000) designations, although it is recognized that the use of Grades or ISO numbers are more contemporary.

Air Cleaning—Since air is one of the greatest potential sources of contaminants in clean rooms, special attention must be given to air drawn into clean rooms by the heating, ventilating, and air conditioning (HVAC) systems. This may be done by a series of treatments that vary somewhat from one installation to another.

In one such series, air from the outside, first, is passed through a prefilter, usually of glass wool, cloth, or shredded plastic, to remove large particles. Then, it may be treated by passage through an electrostatic precipitator. Such a unit induces an electrical charge on particles in the air and removes them by attraction to oppositely charged plates. The air then passes through the most efficient cleaning device, a HEPA filter.

For personnel comfort, air conditioning and humidity control should be incorporated into the system. The latter is also important for certain products, such as those that must be lyophilized, and for the processing of plastic medical devices. The clean, aseptic air is introduced into the Class 100 area and maintained under positive pressure, which prevents outside air from rushing into the aseptic area through cracks, temporarily open doors, or other openings.

Laminar-Flow Enclosures—The required environmental control of aseptic areas has been made possible by the use of laminar airflow, originating through a HEPA filter, occupying one entire side of the confined space. Therefore, it bathes the total space with very clean air, sweeping away contaminants. The orientation for the direction of airflow can be horizontal (Figure 26-8A) or vertical (Figure 26-8B) and may involve a limited area, such as a workbench, or an entire room. Figure 26-9 shows a syringe-filling line in a Grade A/Class 100 area using vertical laminar airflow. The machine is placed in a conventional clean room with vertical LF hood provided through either the ceiling or a LF hood on top of the machine. The machine guarding is a stainless steel frame that can hold the LF hood. The panes are safety glass. This could be an example of a Restricted Access Barrier System (RABS), although there are no gloves installed, thus, requiring doors to be open to access the equipment, which is contrary to the requirements of an authentic RABS. The area outside the RAB can be maintained at a slightly lower level of cleanliness than that inside, perhaps Class 10,000 down to Class 1,000.

Critical areas of processing, wherein the product or product contact surfaces may be exposed to the environment, even for a brief period of time, must meet Class 100 clean room standards.

Any contamination introduced upstream by equipment, arms of the operator, or leaks in the filter will be blown downstream.

### Table 26-4A. Clean Room Classifications

<table>
<thead>
<tr>
<th>European</th>
<th>United States</th>
<th>International Society of</th>
<th>Max No. of Particles per m³</th>
<th>Max No. of Particles per m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade</td>
<td>Classification</td>
<td>Pharm. Eng. Description</td>
<td>&gt;= 0.5 μm</td>
<td>&gt;= 5 μm</td>
</tr>
<tr>
<td>A</td>
<td>100</td>
<td>Critical</td>
<td>3,500</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>Clean</td>
<td>3,500</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>10,000</td>
<td>Controlled</td>
<td>350,000</td>
<td>2,000</td>
</tr>
<tr>
<td>D</td>
<td>100,000</td>
<td>Pharmaceutical</td>
<td>3,500,000</td>
<td>20,000</td>
</tr>
</tbody>
</table>

### Table 26-4B. ISO 14644 Classification of Cleanroom Particle Limits

<table>
<thead>
<tr>
<th>ISO Classification</th>
<th>Maximum Concentration Limits (Particles per Cubic Meter of Air) for Particles &gt;= the Sizes per Each Column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 μm</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>1,000</td>
</tr>
<tr>
<td>4</td>
<td>10,000</td>
</tr>
<tr>
<td>5</td>
<td>100,000</td>
</tr>
<tr>
<td>6</td>
<td>1,000,000</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
</tbody>
</table>
In the instance of horizontal flow, this may be toward the critical working site, the face of the operator, or across the room. Should the contaminant be, for example, penicillin powder, a biohazard material, or viable micro-organisms, the danger to the operator is apparent.

Further, great care must be exercised to prevent cross-contamination from one operation to another, especially with horizontal laminar air flow. For most large-scale operations, as shown in Figure 26-8B and Figure 26-9, a vertical system is much more desirable, with the air flowing through perforations in the countertop or through return louvers at floor level, where it can be directed for decontamination. Laminar-flow environments provide well-controlled work areas, only if proper precautions are observed. Any reverse air currents or movements exceeding the velocity of the HEPA-filtered airflow may introduce contamination, as may coughing, reaching, or other manipulations of the operator. Therefore, laminar-flow work areas should be protected by being located within controlled environments. Personnel should be attired for aseptic processing, as subsequently described. All movements and processes should be planned carefully, to avoid the introduction of contamination upstream of the critical work area. Checks of the air stream should be performed initially and at regular intervals (usually every six months), to be sure no leaks have developed through or around the HEPA filters.

Clean room design, traditionally, has Class 100 rooms adjacent to Class 100,000 rooms. Regulatory authorities have raised great concerns about this significant change in air quality from critical to controlled areas. It is now preferable to have an area classified from Class 1,000 to Class 10,000 in a buffer area between a Class 100 and Class 100,000 area.

Materials Support Area—This area is constructed to withstand moisture, steam, and detergents and is, usually, a Class 100,000 clean room. The ceiling, walls, and floor should be constructed of impervious materials, so moisture runs off and is not held. One of the finishes with a vinyl or epoxy-sealing coat


Figure 26-9. High speed syringe filling machine for pre-sterilized syringes. (Courtesy of Robert Bosch GmbH.)
provides a continuous surface free from all holes or crevices. All such surfaces can be washed at regular intervals to keep them thoroughly clean. These areas should be exhausted adequately, so the heat and humidity are removed for the comfort of personnel. Precautions must be taken to prevent the accumulation of dirt and the growth of micro-organisms due to the high humidity and heat. In this area, preparation for the filling operation, such as cleaning and assembling equipment, is undertaken. Adequate sink and counter space must be provided. This area must be cleanable, and the microbial load must be monitored and controlled. Precautions must also be taken to prevent deposition of particles or other contaminants on clean containers and equipment, until they have been properly boxed or wrapped preparatory to sterilization and depyrogenation.

**Compounding Area**—The formula is compounded in this area. Although it is not essential that this area be aseptic, control of micro-organisms and particulates should be more stringent than in the materials support area. For example, means may need provided to control dust generated from weighing and compounding operations. Cabinets and counters should, preferably, be constructed of stainless steel. They should fit snugly to walls and other furniture, so there are no catch areas dirt can accumulate. The ceiling, walls, and floor should be similar to those for the materials support area.

**Aseptic Area**—The aseptic area requires construction features designed for maximum microbial and particulate control. The ceiling, walls, and floor must be sealed, so they may be washed and sanitized with a disinfectant, as needed. All counters should be constructed of stainless steel and hung from the wall, so there are no legs to accumulate dirt, where they rest on the floor. All light fixtures, utility service lines, and ventilation fixtures should be recessed in the walls or ceiling to eliminate ledges, joints, and other locations for the accumulation of dust and dirt. As much as possible, tanks containing the compounded product should remain outside the aseptic filling area, with the product fed into the area through hose lines. Proper sanitization is required, if the tanks must be moved in. Large mechanical equipment located in the aseptic area should be housed as completely as possible within a stainless steel cabinet, to seal the operating parts and their dirt-producing tendencies from the aseptic environment. Further, all such equipment parts should be located below the filling line. Mechanical parts that will contact the parenteral product should be demountable, so they can be cleaned and sterilized.

Personnel entering the aseptic area should enter only through an airlock. They should be attired in sterile coveralls with sterile hats, masks, goggles, foot covers, and double gloves. Movement within the room should be minimal, and in-and-out movement rigidly restricted during a filling procedure. The requirements for room preparation and the personnel may be relaxed, if the product is to be sterilized terminally in a sealed container. Some are convinced, however, that it is better to have one standard procedure meeting the most rigid requirements.

**Isolation (Barrier) Technology**—Isolator (or barrier) technology has long been used in the pharmaceutical industry and ranges from simple screens to restricted access barriers (RABS) to full isolation systems, all designed to isolate aseptic operations from personnel and the surrounding environment. Sterility tests are now almost exclusively conducted within isolators. A false-positive sterility test is practically unheard of these days, such that, if a positive test does occur, it likely is a true contamination, not as a result of contamination introduced during the test. Isolation technology in various formats has been adapted to automated, large-scale, aseptic filling operations. An example of a sterility test isolator is shown in Figure 26-10, and an example of a filling operation within an isolator is shown in Figure 26-11. The sealed enclosures are presterilized, usually with peracetic acid, hydrogen peroxide vapor, or steam. Sterile supplies are introduced from sterileizable, moveable modules through uniquely engineered transfer ports or directly from attached sterilizers, including autoclaves and hot-air sterilizing tunnels.

Results have been very promising, giving expectation of significantly enhanced control of the aseptic processing environment. Isolators are enclosed, usually positively pressurized units with high efficiency particulate air (HEPA) filters, supplying ISO 5 airflow in a unidirectional manner to the interior. Air recirculates by returning it to the air handlers through sealed ductwork. Cleaning can be manual or automated (clean-in-place). Access to an isolator is through glove ports and sterile transfer systems. Isolators can be located in an ISO 8 or better environment.

The operations are performed within windowed, sealed walls, with operators working through glove ports. The sealed enclosures are presterilized, usually with peracetic acid, hydrogen peroxide vapor, or steam. Sterile supplies are introduced from sterileizable, moveable modules, through uniquely engineered transfer ports or directly from attached sterilizers, including autoclaves and hot-air sterilizing tunnels.

Recent isolator development has been significant, and such systems are now better specified than ever. In advanced aseptic processing facilities, it has been proven that isolators can provide zero colony forming unit (0 cfu) contamination in process operations, whereas the background environment is only at ISO 8 - EC grade D level. However, cost-savings in cleanroom construction and operation may be offset by the construction and validation costs of the isolator system.

For existing production lines, where conversion from conventional filling to filling within an isolator is time and cost...
26-12 features an example of the ‘three bucket’ system used to sanitize facilities. One bucket is to remove as much of the remnant of the ‘dirty’ mop or sponge, the second bucket contains a rinse solution to help clean the mop/sponge, and the third bucket contains the sanitizing solution. The sanitizing solution is exposed during filling and stoppering and the removal of direct human contact with the exposed sterile product. Isolators not only protect the product from potential human contamination, but also protect the human from potential toxic effects of direct exposure to the drug product, especially important for cytotoxic drugs.

**PERSONNEL**

Personnel selected to work on the preparation of a parenteral product must be neat, orderly, and reliable. They should be in good health and free from dermatological conditions that might increase the microbial load. If personnel show symptoms of a head cold, allergies, or similar illness, they should not be permitted in the aseptic area, until recovery is complete. However, a healthy person with the best personal hygiene will still shed large numbers of viable and nonviable particles from body surfaces. This natural phenomenon creates continuing problems, when personnel are present in clean rooms; effective training and proper gowning can reduce, but not eliminate, the problem of particle shedding from personnel.

Aseptic-area operators should be given thorough, formal training in the principles of aseptic processing and the techniques to be employed. Subsequently, the acquired knowledge and skills should be evaluated, to assure that training has been effective, before personnel are allowed to participate in the preparation of sterile products. Retraining should be performed on a regular schedule to enhance the maintenance of the required level of expertise. An effort should be made to imbue operators with an awareness of the vital role they play in determining the reliability and safety of the final product. This is especially true of supervisors, since they should be individuals who not only understand the unique requirements of aseptic procedures, but are able to obtain the full participation of other employees in fulfilling these exacting requirements.

The uniform worn is designed to confine the contaminants discharged from the body of the operator, thereby preventing their entry into the production environment. For use in the
a aseptic area, uniforms should be sterile. Fresh, sterile uniforms should be used after every break period or whenever the individual returns to the aseptic area. In some plants, this is not required, if the product is to be sterilized in its final container. Uniforms, usually, consist of coveralls for both men and women, hoods to cover the hair completely, face masks, and Daeron or plastic boots (Fig. 26-7 and Fig. 26-13). Sterile rubber or latex-free gloves are also required for aseptic operations, preceded by thorough scrubbing of the hands with a disinfectant soap. Two pairs of gloves are put on, one pair at the beginning of the gowning procedure, the other pair after all other apparel has been donned. In addition, gogles are required to complete the coverage of all skin areas.

Dacron or Tyvek uniforms are usually worn, are effective barriers to discharged body particles (viable and nonviable), are essentially lint-free, and are reasonably comfortable. Air showers are sometimes directed on personnel entering the processing area to blow loose lint from the uniforms.

Gowning rooms should be designed to enhance pregowning and gowning procedures by trained operators, so it is possible to ensure the continued sterility of the exterior surfaces of the sterile gowning components. Degowning should be performed in a separate exit room.

**ENVIRONMENTAL CONTROL EVALUATION**

Manufacturers of sterile products use extensive means to control the environment, so these critical injectable products can be prepared free from contamination. Nevertheless, tests should be performed to determine the level of control actually achieved. Normally, the tests consist of counting viable and non-viable particles suspended in the air or settled on surfaces in the workspace. A baseline count, determined by averaging multiple counts, when the facility is operating under controlled conditions, is used to establish the optimal test results expected. During the subsequent monitoring program, the test results are followed carefully for high individual counts, a rising trend, or other abnormalities. If the results exceed selected alert or action levels, a plan of action must be put into operation to determine if or what corrective and follow-up measures are required.

The tests used measure either the particles in a volume of sampled air or the particles settling or present on surfaces. To measure the total particle content in an air sample, electronic particle counters are available, operating on the principle of the measurement of light scattered from particles as they pass through the cell of the optical system. These instruments not only count particles, but also provide a size distribution, based on the magnitude of the light scattered from the particle. Although a volume of air measured by an electronic particle counter will detect all particles instantly, these instruments cannot differentiate between viable (e.g., bacterial and fungal) and non-viable particles. Due to the need to control the level of micro-organisms in the environment in which sterile products are processed, it is also necessary to detect viable particles. These are usually fewer in number than non-viable particles and are only detectable as colony-forming units (CFUs) after a suitable incubation period at, for example, 30°C to 35°C for up to 48 hours. Thus, test results will not be known until 48 hours after the samples are taken, unless more rapid microbial test procedures become dependable and acceptable.

Locations for sampling should be planned to reveal potential contamination levels that may be critical in the control of the environment. For example, the most critical process step is usually the filling of dispensing containers, a site obviously requiring monitoring. In fact, the FDA aseptic processing guidelines require air particle counts be measured during actual filling and closing operations and not more than one foot from the actual work site. Other examples include the gowning room, high-traffic sites in and out of the filling area, the penetration of conveyor lines through walls, and sites near the inlet and exit of the air system.

The sample should be large enough to obtain a meaningful particle count. At sites where the count is expected to be low, the size of the sample may need increased; for example, in Class 100 areas, Whyte and Niven suggest that the sample be at least 30 ft³ and, probably, much more. Many firms employ continuous particle monitoring in Class 100 areas to study trends and/or to identify equipment malfunction.

The slit-to-agar (STA) sampler draws, by vacuum, a measured volume of air through an engineered slit, causing the air to impact the surface of a slowly rotating nutrient agar plate (Fig. 26-14). Micro-organisms adhere to the surface of the agar and grow into visible colonies counted as CFUs, since it is not known whether the colonies arise from a single micro-organism or a cluster.

A widely used method for microbiological sampling consists of the exposure of nutrient agar culture plates to the settling of micro-organisms from the air. This method is very simple and inexpensive to perform, but will detect only those organisms that have settled on the plate; therefore, it does not measure the number of micro-organisms in a measured volume of air (a non-quantitative test). Nevertheless, if the conditions of exposure are repeated consistently, a comparison of CFUs at one sampling site, from one time to another, can be meaningful.

Whyte and Niven suggested that settling plates should be exposed in Class 100 areas for an entire fill (up to 7 to 8 hours), rather than the more common 1 hour. However, excessive dehydration of the medium must be avoided, particularly in the path of laminar-flow air. The European Union GMP guidelines for sterile manufacture of medicinal products suggest an exposure period of not more than four hours that has been adopted by the FDA aseptic processing guidelines.

The number of micro-organisms on surfaces can be determined with nutrient agar plates having a convex surface (Rodac Plates; Fig. 26-15). With these, it is possible to roll the raised agar surface over flat or irregular surfaces to be tested. Organisms are picked up on the agar and grow during subsequent incubation. This method can also be used to assess the number of micro-organisms present on the surface of the uniforms of operators, either as an evaluation of gowning technique, immediately after gowning, or as a measure of the accumulation of micro-organisms during processing. When used, care must be taken to remove any agar residue left on the surface tested.

(Further discussion of proposed, viable particle test methods and the counts accepted can be found in Section <1116> ‘Microbial Evaluation and Classification of Clean Rooms and Other Controlled Environments’ in the USP.)

Results from these tests, although not available until 2 days after sampling, are valuable to keep cleaning, production, and
quality-control personnel apprised of the level of contamination in a given area and, by comparison with baseline counts, will indicate when more-extensive cleaning and sanitizing is needed. The results may also serve to detect environmental control defects, such as failure in air-cleaning equipment or the presence of personnel who may be disseminating large numbers of bacteria without apparent physical ill effects.

Issues regarding environmental monitoring remain among the most controversial aspects of cGMP regulatory inspections of parenteral manufacturing and testing environments. Regulatory trends include requiring an increase in the number and frequency of locations monitored in the clean room and on clean room personnel, enforcing numerical alert and action limits, and linking environmental monitoring data to the decision to release or reject the batch. It has been pointed out that fully gowned personnel still release a finite number of microorganisms (typically 10 to 100 cfu per hour), so it is unreasonable to impose the requirement of zero microbial contamination limits at any location in the clean room.

**MEDIA FILL (PROCESS SIMULATION TESTING)**

FDA inspections have increasingly focused on media fill studies that truly simulate the production process. The media fill or process simulation test involves preparation and sterilization (often by filtration) of sterile trypticase soy broth and filling sterile containers with this broth, under conditions simulating, as closely as possible, those characteristics of a filling process for a product. The key is designing these studies to simulate all factors that occur during the normal production of a lot (Table 26-5).

The media fill provides a ‘one-time’ representation of the capabilities of an aseptic processing operation. Media fills are conducted, when a new filling line or new product container is introduced. For initial qualification of a line or product, three consecutive, separate, and successful media fill runs must take place. The FDA stresses that three is a minimum number of runs. Today, the term ‘successful’ means there is no growth in any of the units filled with sterile broth. All activities and interventions representative of each shift on each line must be simulated during the media fill. All personnel involved in the aseptic filling of a product (i.e., operators, maintenance personnel, microbiology support personnel) must participate in at least one media fill run per year. Typically, for each filling line

<table>
<thead>
<tr>
<th>Table 26-5. Factors to Consider in the Design of Media Fill Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Duration of longest run</td>
</tr>
<tr>
<td>• Worst-case environmental conditions</td>
</tr>
<tr>
<td>• Number and type of interventions, stoppages, adjustments, transfers</td>
</tr>
<tr>
<td>• Aseptic assembly of equipment</td>
</tr>
<tr>
<td>• Number and activities of personnel</td>
</tr>
<tr>
<td>• Number of aseptic additions</td>
</tr>
<tr>
<td>• Shift breaks, changes, multiple gownings</td>
</tr>
<tr>
<td>• Number/type of aseptic equipment disconnections and connections</td>
</tr>
<tr>
<td>• Aseptic samples</td>
</tr>
<tr>
<td>• Line speed/configuration</td>
</tr>
<tr>
<td>• Manual weight checks</td>
</tr>
<tr>
<td>• Operator fatigue</td>
</tr>
<tr>
<td>• Container/closure types run on the line</td>
</tr>
<tr>
<td>• Temperature/relative humidity extremes</td>
</tr>
<tr>
<td>• Conditions permitted before line clearance</td>
</tr>
<tr>
<td>• Container/closure surfaces which contact formulation during aseptic process</td>
</tr>
</tbody>
</table>

**Figure 26-14.** Examples of a Slit-To-Agar (STA) Quantitative Air Sampler.

**Figure 26-15.** Example of a Rodac plate. (Courtesy of Baxter Healthcare Corporation.)
and process, the filling operation is validated for the smallest and largest container size that will be used.

After initial qualification, media fills are then conducted on a periodic basis, usually twice a year on the same filling line, to ensure that conditions that existed during the initial qualification have been maintained. For periodic qualification, only one successful media fill run is required. If any media fill run fails or significant changes occur with the line, facility, or personnel, then the initial qualification media fill (three consecutive successful runs) must be conducted. Any changes in the process must be evaluated for its level of significance (change control quality system) that would necessitate a media fill validation run. Any media fill failure must be thoroughly investigated and followed by multiple repeat media fill runs. It is considered inappropriate to ‘invalidate’ a media fill run.

The number of containers filled with media, ideally, should be the same as the actual number filled, according to the batch record for the product being validated. Of course, this is unrealistic for large batch sizes. Therefore, the number of units filled must be sufficient to reflect the effects of all worst case filling rates. For example, operator fatigue and the maximum number of interventions and stoppages may be incorporated into the media fill protocol. When media filling first started, the acceptable rate of positives (number of containers that showed contamination after incubating the culture media) was 1 out of 1000 (0.1%). Later that number became 1 out of 3000 to account for 95% confidence of a contamination rate of 0.1%. Today, 1 positive out of 3000 is no longer acceptable. Table 26-6 presents that ISO standard used to determine the minimum number of containers filled with media and the acceptable number of positives. The most common number of containers filled with media in the industry is 4750 with three consecutive runs of 4750 used for initial performance qualification of a new product and/or filling/closing line. This same number of units filled—4750—is also used for the routine semi-annual requalification media fills. The expected number of positive media fills (growth seen upon incubation) is zero. One or more failures likely means there is a significant breach in the aseptic manufacturing process, and the ensuing investigation must do everything possible to find the assignable cause.

After filling with culture media, but prior to incubation, all units should be inverted or swirled to enable the media to make contact with all internal surfaces of the container/closure system.

The culture media used for each media fill exercise must be tested to ensure it will support the growth of micro-organisms, if they are present. Challenge organisms used in the media challenge pretesting should include those isolated from environmental/personnel monitoring, those isolated from positive sterility test results and USP growth promotion micro-organisms. The positive control units inoculated with approximately 100 CFUs of these challenge organisms are incubated at temperatures and times validated to show microbial growth if present. After the 14 day incubation period of the media fill containers, negative control units should then be inoculated with challenge organisms, to prove the media will still support growth, if present.

Inspection of media filled units, before and after incubation, is conducted by individuals trained as qualified inspectors and certified by the quality control unit. It is permissible that any unit, after filling, that is found to lack integrity be rejected from being part of the media fill incubation, just as a product vial would be rejected if a critical defect were found. However, if a media fill unit is found damaged after incubation is underway, it must remain incubated and counted in the data for the media fill batch. Procedures must be very clear and specific regarding samples taken during the media fill that simulate the actual sampling process and why these units are not part of those incubated.

Other requirements of a valid media fill experiment include:

- Must have the appropriate criteria for batch yield and accountability, just like a product batch.
- Must identify any contaminant to the species level and perform complete investigations of failed media fills.
- FDA advocates videotape media fills to identify personnel practices that could negatively impact the aseptic process.
- Media fill duration, according to FDA, EU, ISO, CEN (European Committee of Standardization), and PIC, must be sufficiently long to include all required manipulations and cover the same length of time normally consumed by the commercial process. Most media fills are a minimum of 3 hours; some may be as long as 24 hours.

## PRODUCTION PROCEDURES

The processes required for preparing sterile products constitute a series of events initiated with the procurement of approved raw materials (drugs, excipients, vehicles, etc.) and primary packaging components (containers, closures, etc.) and ending with the sterile product sealed in its dispensing package. Each step in the process must be controlled very carefully, so the product has its required quality. To ensure the latter, each process should be validated to ensure it is accomplishing what it is intended to do. For example, an autoclave sterilization process must be validated by producing data showing it effectively kills resistant forms of micro-organisms; or, a cleaning process for rubber closures should provide evidence that it is cleaning closures to the required level of cleanliness; or, a filling process should provide evidence that it repeatedly delivers the correct fill volume per container. The validation of processes requires extensive and intensive effort to be successful and is an integral part of cGMP requirements.

## CLEANING CONTAINERS AND EQUIPMENT

Containers and equipment coming in contact with parenteral preparations must be cleaned meticulously. It should be obvious that even new, unused containers and equipment are contaminated with such debris as dust, fibers, chemical films, and other materials arising from such sources as the atmosphere, cartons, the manufacturing process, and human hands. Residues from previous use must be removed from used equipment, before it is suitable for reuse. Equipment should be reserved exclusively for use only with parenteral preparations and, where conditions dictate, only for one product to reduce the risk of contamination. For many operations, particularly with biologic and biotechnology products, equipment is dedicated for only one product.

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**Table 26-6. ISO 13408-1 Standards for Minimum Number of Containers Filled with Media and the Acceptable Number of Positives**

<table>
<thead>
<tr>
<th>Number of Media Fill Units</th>
<th>Allowable Number of Failed Units (95% C.L.) by ISO</th>
<th>Allowable Number of Failed Units by Simple Math</th>
</tr>
</thead>
<tbody>
<tr>
<td>3,000</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>4,750</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>6,300</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>7,760</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>9,160</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>10,520</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>11,850</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>13,150</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>14,440</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>15,710</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>16,970</td>
<td>11</td>
<td>17</td>
</tr>
</tbody>
</table>

Sample chapter from *Remington: Essentials of Pharmacuetics*
A variety of machines are available for cleaning new containers for parenteral products. These vary in complexity from a small, hand loaded, rotary rinser to large, automatic washers capable of processing several thousand containers per hour. The selection of the particular type is determined largely by the physical type of containers, the type of contamination, and the number to be processed in a given period of time.

Validation of cleaning procedures for equipment is another ‘hot topic,’ with respect to cGMP regulatory inspections. Inadequate cleaning processes have been a frequent citing by FDA and other regulatory inspectors, when inspecting both active ingredient and final product manufacturing facilities. It is incumbent upon parenteral manufacturers to establish scientifically justified acceptance criteria for cleaning validation. If specific analytical limits for target residues are arbitrarily set, this will cause concern for quality auditors. Validation of cleaning procedures can be relatively complicated, due to issues with sample methods (e.g., swab, final rinse, and testing of subsequent batch), sample locations, sensitivity of analytical methods, and calculations used to establish cleaning limits. Cleaning validation involves challenging the ‘hardest to clean’ surfaces with a well-defined sample, typically an active pharmaceutical ingredient (API) or a known ‘hard to remove’ substance like a sparingly soluble pharmaceutical or ‘sticky’ protein. The cleaning procedure is applied, using either swab samples or rinse samples obtained from the ‘hardest to clean’ surface locations. These samples are analyzed for residual API, using either a specific analytical technique, such as high performance liquid chromatography, or non-specific method, such as total organic carbon. Acceptance limits must be justified that the cleaning procedure accomplishes repeatedly before the cleaning method can be considered valid. (Additional discussion of this topic is found in Chapter 25 – Sterilization Processes and Sterility Assurance.)

**CHARACTERISTICS OF MACHINERY**

Regardless of the type of cleaning machine selected, certain fundamental characteristics are usually required:

1. The liquid or air treatment must be introduced in such a manner that it will strike the bottom of the inside of the inverted container, spread in all directions, and smoothly flow down the walls and out the opening with a sweeping action. The pressure of the jet stream should be such that there is minimal splashing and turbulence inside. Splashing may prevent cleaning all areas, and turbulence may redeposit loosened debris. Therefore, direct introduction of the jet stream within the container with control of its flow is required.

2. The container must receive a concurrent outside rinse.

3. The cycle of treatment should provide a planned sequence, alternating very hot and cool treatments. The final treatment should be an effective rinse with WFI.

4. All metal parts coming in contact with the containers and with the treatments should be constructed of stainless steel or some other non-corroding and non-contaminating material.

**TREATMENT CYCLE**

The cycle of treatments to be employed varies with the condition of the containers to be cleaned. In general, loose debris can be removed by vigorous rinsing with water. Detergents are rarely used for new containers, due to the risk of leaving detergent residues. However, a thermal-shock sequence in the cycle is usually employed to aid, by expansion and contraction, loosening of debris that may be adhering to the container wall. Sometimes, only an air rinse is used for new containers, if only loose debris is present. In all instances, the final rinse, whether air or WFI, must be ultraclean, so no particulate residues are left by the rinsing agent.

Only new containers are used for parenterals. Improvements have been made in maintaining their cleanliness, during shipment from the manufacturer through tight, low-shedding packaging, including plastic blister packs.

**MACHINERY FOR CONTAINERS**

The machinery available for cleaning containers embodies the previously mentioned principles, but varies in the mechanics by which it is accomplished. In one manual loading type, the jet tubes are arranged on arms like the spokes of a wheel, which rotate around a center post through which the treatments are introduced. An operator places the unclean containers on the jet tubes, as they pass the loading point, and removes the clean containers as they complete one rotation. A continuous automated line operation, capable of cleaning hundreds of containers per hour, is shown in Figure 26-16. The vials are fed into the rotary rinser in the foreground, transferred automatically to the covered sterilizing tunnel in the center, conveyed through the wall in the background, and discharged into the filling clean room.

**HANDLING AFTER CLEANING**

The wet, clean containers must be handled in such a way that contamination is not reintroduced. A wet surface will collect contaminants much more readily than a dry surface will. For this reason, wet, rinsed containers must be protected (e.g., by a laminar flow of clean air until covered, within a stainless steel box, or within a sterilizing tunnel). In addition, microorganisms are more likely to grow in the presence of moisture. Therefore, wet, clean containers should be dry-heat sterilized, as soon as possible after washing. Doubling the heating period is also adequate to destroy pyrogens; for example, increasing the dwell time at 250°F from 1 to 2 hours, however, the actual time-temperature conditions required must be validated.

Increases in process rates have necessitated the development of continuous, automated line processing, with a minimum of individual handling, still maintaining adequate control of the cleaning and handling of the containers. The clean, wet containers are protected by filtered, laminar-flow air from the rinsing, through the tunnel, and until they are delivered to the filling line (Fig. 26-16).

**CLOSURES**

The rough, elastic, and convoluted surface of rubber closures renders them difficult to clean. In addition, any residue of lubricant from molding or surface ‘bloom’ of inorganic constituents must be removed. The normal procedure calls for gentle agitation in a hot solution of a mild water softener or
detergent. The closures are removed from the solution and rinsed several times, or continuously for a prolonged period, with filtered WFI. The rinsing is done in a manner that flushes away loosened debris. The wet closures are carefully protected from environmental contamination, sterilized, usually by steam sterilization (autoclaving), and stored in closed containers, until ready for use. This cleaning and sterilizing process must also be validated with respect to rendering the closures free from pyrogens. Actually, it is the cleaning and final, thorough rinsing with WFI that must remove pyrogens, since autoclaving does not destroy pyrogens. If the closures were immersed during autoclaving, the solution is drained off, before storage, to reduce hydration of the rubber compound. If the closures must be dry for use, they may be subjected to vacuum drying at a temperature in the vicinity of 100°C. Some freeze-dried products require extremely dry closures to avoid desorption of moisture from the closure into the moisture-sensitive powder during storage. This may require drying times of hours, following steam sterilization.

The equipment used for washing large numbers of closures is usually an agitator or horizontal basket-type automatic washing machine. Due to the risk of particulate generation from the abrading action of these machines, some procedures simply call for heating the closures in kettles in detergent solution, followed by prolonged flush rinsing. The final rinse should always be with low-particulate WFI. Figure 26-17 shows an example of a modern closure processor that washes, siliconizes, sterilizes, and transports closures directly to the filling line.

It is also possible to purchase rubber closures already cleaned and lubricated in sterilizable bags supplied by the rubber closure manufacturer.

**EQUIPMENT**

All equipment should be disassembled as much as possible to provide access to internal structures. Surfaces should be scrubbed thoroughly with a stiff brush, using an effective detergent and paying particular attention to joints, crevices, screw threads, and other structures where debris is apt to collect. Exposure to a stream of clean steam aids in dislodging residues from the walls of stationary tanks, spigots, pipes, and similar structures. Thorough rinsing with distilled water should follow the cleaning steps.

Due to the inherent variation in manual cleaning, the difficult accessibility of large stationary tanks, and the need to validate the process, computer-controlled systems, usually automated, known as clean-in-place (CIP) systems, have been developed. Such an approach involves designing the system, normally of stainless steel, with smooth, rounded internal surfaces and without crevices. That is, for purpose, with welded, rather than threaded, connections. Cleaning is accomplished with the scrubbing action of high-pressure spray balls or nozzles delivering hot detergent solution from tanks captive to the system, followed by thorough rinsing with WFI. The system is often extended to allow sterilizing-in-place (SIP), to accomplish sanitizing or sterilizing as well.

Rubber tubing, rubber gaskets, and other rubber parts may be washed in a manner as described for rubber closures. Thorough rinsing of tubing must be done by passing WFI through the tubing lumen. However, due to the relatively porous nature of rubber compounds and the difficulty in removing all traces of chemicals from previous use, it is considered by some inadvisable to reuse rubber or polymeric tubing. Rubber tubing must be left wet when preparing for sterilization by autoclaving.

**PRODUCT PREPARATION**

The basic principles employed in the compounding of the product are essentially the same as those used, historically, by pharmacists. However, large-scale production requires appropriate adjustments in the processes and their control.

A master formula should be developed and be on file. Each batch formula sheet should be prepared from the master and confirmed for accuracy. All measurements of quantities should be made as accurately as possible and checked by a second qualified person. Frequently, formula documents are generated by computer, and the measurements of quantities of ingredients are computer controlled. Although most liquid preparations are dispensed by volume, they are prepared by weight, since weighing can be performed more accurately than volume measurements, and no consideration needs to be given to the temperature.
Care must be taken that equipment is not wet enough to dilute the product significantly or, in the case of anhydrous products, to cause a physical incompatibility. The order of mixing of ingredients may affect the product significantly, particularly those of large volume, where attaining homogeneity requires considerable mixing time. For example, the adjustment of pH by the addition of an acid, even though diluted, may cause excessive local reduction in the pH of the product, so adverse effects are produced before the acid can be dispersed throughout the entire volume of product.

Parenteral dispersions, including colloids, emulsions, and suspensions, provide particular problems. In addition to the problems of achieving and maintaining proper reduction in particle size under aseptic conditions, the dispersion must be kept in a uniform state of suspension throughout the preparative, transfer, and subdividing operations.

Biopharmaceuticals are usually extremely sensitive to many environmental and processing conditions exposed to during production, such as temperature, mixing time and speed, order of addition of formulation components, pH adjustment and control, and contact time with various surfaces, such as filters and tubing. Development studies must include evaluation of manufacturing conditions to minimize adverse effects of the process on the activity of the protein. Among many causes for protein aggregation are protein particles, resulting from heterogeneous nucleation on foreign micro- or nanoparticles, originating from the manufacturing process (i.e., mixing tanks, process tubing, filter systems, filling machines) or any other stainless steel, rubber, glass, or plastic surface) and from the container/closure system. It is well known that silicone oil, used as a lubricant for rubber closures, on vials, on rubber plungers, in prefilled syringes, and to coat the inner surface of glass syringes and cartridges can also induce protein aggregation. Although switching from silicone-coated containers to plastic containers and using coated rubber closures, rather than siliconized rubber closures, may minimize or eliminate the problems of protein aggregation, other challenges surface, such as unknown leachate potential, sterilization of components, vendor reliability, and cost.

The formulation of a stable product is of paramount importance. Certain aspects of this are mentioned in the discussion of components of the product. Excessive coverage of the topic is not possible within the limits of this text, but further coverage is provided in Chapters 24 [Solutions, Emulsions, Suspensions and Extracts] and 4 [Stability of Pharmaceutical Products]. It should be mentioned here, however, that the thermal sterilization of parenteral products increases the possibility of chemical reactions. Such reactions may progress to completion, during the period of elevated temperature in the autoclave, or be initiated at this time but continue during subsequent storage. The assurance of attaining product stability requires a high order of pharmaceutical knowledge and responsibility.

Filtration

After a product has been compounded, it must be filtered, if it is a solution. The primary objective of filtration is to clarify the solution. A further step, removing particulate matter down to 0.2 μm in size, would eliminate micro-organisms and would accomplish cold sterilization. A solution with a high degree of clarity conveys the impression of high quality and purity, desirable characteristics for a parenteral solution.

Filters are thought to function by one or, usually, a combination of: 1) sieving or screening, 2) entrapment or impaction, and 3) electrostatic attraction (Fig. 26-18). When a filter retains particles by sieving, they are retained on the surface of the filter. Entrapment occurs when a particle smaller than the dimensions of the passageway (pore) becomes lodged in a turn or impacted on the surface of the passageway. Electrostatic attraction causes particles opposite in charge to that of the surface of the filter pore to be held or adsorbed to the surface. It should be noted that increasing, prolonging, or varying the force behind the solution may tend to sweep particles initially held by entrapment or electrostatic charge through the pores and into the filtrate.

Membrane filters are used exclusively for parenteral solutions, due to their particle-retention effectiveness, non-shedding property, non-reactivity, and disposable characteristics. However, it should be noted that non-reactivity does not apply in all cases. For example, polypeptide products may show considerable adsorption through some membrane filters, but those composed of polysulphone and polyvinylidine difluoride (PVDF) have been developed to be essentially non-adsorptive for these products. The most common membranes are composed of Cellulose esters, Nylon, Polysulphone, Polycarbonate, PVDF, or Polytetrafluoroethylene (Teflon).

Filters are available as flat membranes or pleated into cylinders (Fig. 26-19) to increase surface area and, thus, flow rate. Each filter in its holder should be tested for integrity before and after use, particularly, if it is being used to eliminate micro-organisms. This integrity test is performed either as the ‘bubble-point test’ or as the ‘diffusion or forward flow’ test. The bubble point test is commonly used on smaller filters. As the surface area of filters becomes large, diffusion of air through the water-filled pores tends to obscure the bubble point. Therefore, the diffusion test has been developed as an integrity test for filters with large surface areas. A ‘pressure hold test’ can also be applied to large surface area filters. The filter manufacturer will recommend the best integrity test for the filter system in question.

These are tests to detect the largest pore or other opening through the membrane. The basic test is performed by gradually raising air pressure on the upstream side of a water-wet filter. The bubble point is the pressure obtained when air bubbles first appear downstream of the filter. The diffusion or forward flow test raises pressure to some point below the known bubble point pressure, then diffusion flow (usually in mL/min) is measured. These pressures are characteristic for each pore size of a filter and are provided by the filter manufacturer. For example, a 0.2-μm cellulose ester filter will bubble at about 50 psig or a diffusive flow rating of no greater than 13 mL/min at a pressure of 40 psig. If the filter is wetted with other liquids, such as a product, the bubble point will differ and must be determined experimentally. If the bubble point is lower than the rated
During the filling of containers with a product, the most stringent requirements must be exercised to prevent contamination, particularly if the product has been sterilized by filtration and will not be sterilized in the final container. Under the latter conditions, the process is called an 'aseptic fill' and is validated by providing a HEPA-filtered aseptic environment, equipment, and manipulative technique of the operator. Until it can be sealed in the container. Therefore, this operation is carried out with a minimum exposure time, even though maximum protection is provided by filling under a blanket of HEPA-filtered laminar-flow air within the aseptic area.

Most frequently, the compounded product is in the form of a liquid. However, products are also compounded as dispersed systems (e.g., suspensions and emulsions) and as powders. A liquid is more readily subdivided uniformly and introduced into a container having a narrow mouth than is a solid. Mobile liquids are considerably easier to transfer and subdivide than viscous, sticky liquids, which require heavy-duty machinery for rapid production filling.

Although many devices are available for filling containers with liquids, certain characteristics are fundamental to them all. A means is provided for repetitively forcing a measured volume of the liquid through the orifice of a delivery tube introduced into the container. The size of the delivery tube will vary from that of about a 20-gauge hypodermic needle to a tube 1/2 in or more in diameter. The size required is determined by the physical characteristics of the liquid, the desired delivery speed, and the inside diameter of the neck of the container. The tube must enter the neck and deliver the liquid well into the neck to eliminate spillage, allowing sufficient clearance to avoid air to leave the container as the liquid enters. The delivery tube should be as large in diameter as possible to reduce resistance and decrease velocity of flow of the liquid. For smaller volumes of liquids, delivery usually is obtained from the stroke of the plunger of a syringe, forcing the liquid through a two-way valve, providing for alternate filling of the syringe and delivery of mobile liquids. For heavy, viscous liquids, a sliding piston valve, the turn of an auger in the neck of a funnel, or the oscillation of a rubber diaphragm may be used. Also, stainless steel syringes are required with viscous liquids, because glass syringes are not strong enough to withstand the high pressures developed during delivery. For large volumes, the quantity delivered is measured in the container by the level of fill in the container, the force required to transfer the liquid being provided by gravity, a pressure pump, or a vacuum pump.

The narrow neck of an ampoule limits the clearance possible between the delivery tube and the inside of the neck. Since a drop of liquid normally hangs at the tip of the delivery tube after a delivery, the neck of an ampoule will be wet as the delivery tube is withdrawn, unless the drop is retracted. Therefore, filling machines should have a mechanism by which this drop can be drawn back into the lumen of the tube. Since the liquid will be in intimate contact with the parts of the machine through which it flows, these must be constructed of non-reactive materials, such as borosilicate glass or stainless steel. In addition, they should easily be demountable for cleaning and sterilization.

Figure 26-20. Syringe filling machine. (Courtesy Baxter Healthcare Corporation.)

There are three main methods for filling liquids into containers with high accuracy: volumetric filling, time/pressure dosing, and net weight filling. Volumetric filling machines, employing pistons or peristaltic pumps, are most commonly used.

When high-speed filling rates are desired but accuracy and precision must be maintained, multiple filling units are often joined in an electronically coordinated machine (Fig. 26-20 and Fig. 26-21). When the product is sensitive to metals, a peristaltic-pump filler may be used, because the product comes in contact only with silicone rubber tubing. However, this sacrifices filling accuracy.

Time-pressure (or time-gravity) filling machines are gaining popularity in filling sterile liquids. A product tank is connected to the filling system equipped with a pressure sensor. The sensor continuously measures pressure and transmits values to the PLC system controlling the flow of product from tank to filling manifold. Product flow occurs when tubing is mechanically unpinched and stops when tubing is mechanically pinched. The main advantage of time/pressure filling operations is that these filling apparatuses do not contain mechanical moving parts in the product stream. The product is driven by pressure (usually nitrogen) with no pumping mechanism involved. Thus, especially for proteins that are quite sensitive to shear forces, time/pressure filling is preferable.

Most high-speed fillers for large-volume solutions use the bottle as the measuring device, transferring the liquid either by vacuum or by positive pressure from the bulk reservoir to the individual unit containers. Therefore, a high accuracy of fill is not achievable.

The USP requires that each container be filled with a sufficient volume in excess of the labeled volume to ensure withdrawal of the labeled volume and provides a table of suggested fill volumes.

The filling of a small number of containers may be accomplished with a hypodermic syringe and needle, the liquid drawn into the syringe and forced through the needle into the container. An example of such a device that provides greater speed of filling is the Cornwall Pipet (Becton Dickinson). The device has a two-way valve between the syringe and the needle and a means for setting the stroke of the syringe, so the same volume is delivered each time. Clean, sterile, disposable assemblies operating on the same principle have particular usefulness in hospital pharmacy or experimental operations.
SOlids

Sterile solids, such as antibiotics, are more difficult to subdivide evenly into containers than are liquids. The rate of flow of solid material is slow and often irregular. Even though a container with a larger-diameter opening is used to facilitate filling, it is difficult to introduce the solid particles, and the risk of spillage is ever-present. The accuracy of the quantity delivered cannot be controlled, as well as with liquids. Due to these factors, the tolerances permitted for the content of such containers must be relatively large.

Some sterile solids are subdivided into containers by individual weighing. A scoop is usually provided to aid in approximating the quantity required, but the quantity filled into the container is finally weighed on a balance. This is a slow process. When the solid is obtainable in a granular form, so it will flow more freely, other methods of filling may be employed. In general, these involve the measurement and delivery of a volume of the granular material that has been calibrated in terms of the weight desired. In the machine shown in Figure 26-22, an adjustable cavity in the rim of a wheel is filled by vacuum and

Figure 26-21. Vial filling machine, distant and close-up views. (Courtesy, Baxter Healthcare Corporation.)

Figure 26-22. Perry Accofil Sterile Powder Filling Machine. A. Principle of operation. B. Filler inside a barrier system. C. Close-up view of filler. (Courtesy of M&O Perry Industries, Inc.)
the contents held by vacuum, until the cavity is inverted over the container. The solid material is then discharged into the container by a puff of sterile air.

### SEALING

#### AMPOULES

Filled containers should be sealed as soon as possible, to prevent the contents from being contaminated by the environment. Ampoules are sealed by melting a portion of the glass neck. Two types of seals are employed normally: tip-seals (bead-seals) or pull-seals.

Tip-seals are made by melting enough glass at the tip of the neck of an ampoule to form a bead and close the opening. These can be made rapidly in a high-temperature gas-oxygen flame. To produce a uniform bead, the ampoule neck must be heated evenly on all sides, such as by burners on opposite sides of stationary ampoules or by rotating the ampoule in a single flame. Care must be taken to properly adjust the flame temperature and the interval of heating results in the expansion of the gases within the ampoule against the soft bead seal, which causes a bubble to form. If the bubble bursts, the ampoule is no longer sealed; if it does not, the wall of the bubble will be thin and fragile. Insufficient heating will leave an open capillary through the center of the bead. An incompletely sealed ampoule is called a 'leaker'.

Pull-seals are made by heating the neck of the ampoule below the tip, leaving enough of the tip for grasping with forceps or other mechanical devices. The ampoule is rotated in the flame from a single burner. When the glass has softened, the tip is grasped firmly and pulled quickly away from the body of the ampoule, which continues to rotate. The small capillary tube, thus, formed is twisted closed. Pull-sealing is slower, but the seals are more secure than tip-sealing. Figure 26-23 shows a machine combining the steps of filling and pull-sealing ampoules.

Ampoules having a wide opening must be sealed by pull-sealing. Fracture of the neck of ampoules during sealing may occur, if wetting of the necks occurs at the time of filling. Also, wet necks increase the frequency of bubble formation and unsightly carbon deposits, if the product is organic.

To prevent decomposition of a product, it is sometimes necessary to displace the air in the space above the product in the ampoule with an inert gas, by introducing a stream of the gas, such as nitrogen or carbon dioxide, during or after filling with the product. Immediately thereafter, the ampoule is sealed, before the gas can diffuse to the outside. This process should be validated to ensure adequate displacement of air by the gas in each container.

**Figure 26-23.** FPS2CA Automatic Monoblock Closed Ampule Filling and Sealing Machine. (Courtesy of and with permission from Cozzoli Machine Company.)

### VIALS AND BOTTLES

Glass or plastic vials and bottles are sealed by closing the opening with a rubber closure (stopper). This must be accomplished as rapidly as possible after filling and with reasoned care, to prevent contamination of the contents. The large opening makes the introduction of contamination much easier than with ampoules. Therefore, during the critical exposure, the open containers should be protected from the ingress of contamination, preferably with a blanket of HEPA-filtered laminar airflow.

The closure must fit the mouth of the container snugly enough, so its elasticity seals rigid to slight irregularities in the lip and neck of the container. However, it must not fit so snugly that it is difficult to introduce into the neck of the container. Preferably, closures are inserted mechanically, using an automated process, especially with high-speed processing. To reduce friction, so the closure may slide more easily through a chute and into the container opening, the closure surfaces are halogenated or treated with silicone. When the closure is positioned at the insertion site, it is pushed mechanically into the container opening (Fig. 26-24). When small lots are encountered, manual stoppering with forceps may be used, but such a process poses greater risk of introducing contamination than automated processes. This is a good test for evaluation of operator aseptic techniques, but not recommended for any product filling and stoppering.

Container-closure integrity testing has become a major focus for the industry, due to emphasis by regulatory agencies. Container-closure integrity measures the ability of the seal between the glass or plastic container opening and the rubber closure to remain tight and fit and to resist any ingress of microbial contamination during product shelf life. Container-closure integrity test requirements are covered in USP <1207>, and the various test methods are described by Guazzo.

Rubber closures are held in place by means of aluminum caps. The caps cover the closure, crimped under the lip of the vial or bottle to hold them in place. The closure cannot be removed without destroying the aluminum cap; it is tamperproof. Therefore, an intact aluminum cap is proof that the closure has not been removed intentionally or unintentionally. Such confirmation is necessary to ensure the integrity of the contents, as to sterility and other aspects of quality.

The aluminum caps are designed so the outer layer of double-layered caps, or the center of single-layered caps, can be removed to expose the center of the rubber closure, without disturbing the band that holds the closure in the container. Rubber closures for use with intravenous administration sets often have a permanent hole through the closure. In such cases, a thin rubber disk, overlaid with a solid aluminum disk, is

**Figure 26-24.** Mechanical device for inserting rubber closures in vials. (Courtesy of Baxter Healthcare Corporations.)
placed between an inner and outer aluminum cap, thereby providing a seal of the hole through the closure.

Single-layered aluminum caps may be applied using a hand crimper. Double- or triple-layered caps (Fig. 26-25) require greater force for crimping; therefore, heavy-duty mechanical crimpers (Fig. 26-26) are required.

**STERILIZATION**

Whenever possible, the parenteral product should be sterilized, after being sealed in its final container (terminal sterilization) and within as short a time as possible after filling and sealing are completed. Since this usually involves a thermal process, although there is a trend in applying radiation sterilization to finished products, due consideration must be given to the effect of the elevated temperature, upon the stability of the product. Many products, both pharmaceutical and biological, are affected adversely by elevated temperatures required for thermal sterilization. Heat-labile products must, therefore, be sterilized by a non-thermal method, usually by filtration through bacteria-retaining filters. Subsequently, all operations must be carried out in an aseptic manner, so contamination is not introduced into the filtrate. Colloids, oleaginous solutions, suspensions, and emulsions that are thermolabile may require a process in which each component is sterilized separately and the product is formulated and processed under aseptic conditions.

The performance of an aseptic process is challenging, but technical advances in aseptic processing, including improved automation, use of isolator systems, formulations to include antimicrobial effects, and combinations of limited sterilization with aseptic processing, have decreased the risk of contamination. Therefore, the successes realized should encourage continued efforts to improve the assurance of sterility achievable with aseptic processing. The importance of this is that, for many drug solutions and essentially all biopharmaceutical products, aseptic processing is the only method that can be considered for preparing a sterile product.

Interaction among environmental conditions, the constituents in the closure, and the product may result in undesirable closure changes, such as increased brittleness or stickiness, which may cause loss of container-closure seal integrity. Thus, shelf life integrity is an important consideration in closure selection and evaluation.

The assessment of aseptic-processing performance is based on the contamination rate resulting from periodic process simulations using media-filling, instead of product-filling of containers. A contamination rate no greater than 0.1% at 95% confidence has been considered indicative of satisfactory performance in the industry. However, with current advances in aseptic processing capabilities, lower contamination rates may be achievable.

Radiation sterilization, as mentioned, is gaining momentum as an alternative terminal sterilization method. There has been limited understanding of the molecular transformations that may occur in drug molecules and excipients under exposure to the high-energy gamma radiation levels of the process. However, lower energy beta particle (electron beam) radiation has seen some success. Significant research must still be accomplished, before radiation sterilization is used as a terminal sterilization process. The use of radiation for the sterilization of materials, such as plastic medical devices, is well established.

Dry-heat sterilization may be employed for a few dry solids not affected adversely by the high temperatures and for the relatively long heating period required. This method is applied most effectively to the sterilization of glassware and metalware. After sterilization, the equipment will be sterile, dry, and, if the sterilization period is long enough, pyrogen-free.

Saturated steam under pressure (autoclaving) is the most commonly used and the most effective method for the sterilization of aqueous liquids or substances that can be reached or penetrated by steam. A survival probability of at least $10^{-6}$ is readily achievable with terminal autoclaving of a thermally stable product.
Figure 26-27. An example of a modern autoclave for sterilization. (Courtesy of Getinge.)

However, it needs noted that, for terminal sterilization, the assurance of sterility is based on an evaluation of the lethality of the process (i.e., of the probable number of viable microorganisms remaining in product units). However, for aseptic processing, where the components used have been sterilized separately by validated processes and aseptically put together, the level of sterility assurance is based on an evaluation of the probable number of product units contaminated during the process.

Figure 26-27 shows an example of a modern autoclave for sterilization. Since the temperature employed in an autoclave is lower than that for dry-heat sterilization, equipment made of materials, such as rubber and polypropylene, may be sterilized, if the time and temperature are carefully controlled. As mentioned previously, some injections are affected adversely by the elevated temperature required for autoclaving. For some products, such as Dextrose Injection, a shortened cycle, using an autoclave designed to permit a rapid temperature rise and rapid cooling with water spray or other cooling methods, makes it possible to use this method. It is ineffective in anhydrous conditions, such as within a sealed ampoule containing a dry solid or an anhydrous oil. Other products that will not withstand autoclaving temperatures may withstand marginal thermal methods, such as tyndallization or pasteurization (e.g., 10 to 12 hours at 60°C). These methods may be rendered more effective for some injections, by the inclusion of a bacteriostatic agent in the product.

Articles to be sterilized must be properly wrapped or placed in suitable containers to permit penetration of sterilants and provide protection from contamination after sterilization. Sheets or bags made of special steam-penetrating paper or polymeric materials are available for this purpose. Further, containers or bags impervious to steam can be equipped with a microbe-excluding vent filter to permit adequate steam penetration and air exit. Multiple wrapping permits sequential removal of outer layers, as articles are transferred from zones of lower to higher environmental quality. The openings of equipment subjected to dry-heat sterilization are often covered with metal or glass covers. Laboratories often used silver-aluminum foil for covering glassware used for endotoxin testing. Wrapping materials commonly used for steam sterilization may be combustible or otherwise become degraded under dry-heat sterilization conditions.

The effectiveness of any sterilization technique must be proved (validated), before it is employed in practice. Since the goal of sterilization is to kill micro-organisms, the ideal indicator to prove the effectiveness of the process is a resistant form of an appropriate micro-organism, normally resistant spores (a biological indicator, or BI). Therefore, during validation of a sterilization process, BIs of known resistance and numbers are used in association with physical-parameter indicators, such as recording thermocouples. Once the lethality of the process is established in association with the physical measurements, the physical measurements can be used for subsequent monitoring of in-use processes without the BIs. Eliminating the use of BIs in direct association with human-use products is appropriate, due to the ever-present risk of an undetected, inadvertent contamination of a product or the environment. The number of spores and their resistance in BIs used for validation studies must be accurately known or determined. Additionally, the manner in which BIs are used in validation is critical and must be controlled carefully.

In addition to the data printout from thermocouples, sometimes other physical indicators are used, such as color-change and melting indicators, to give visual indication that a package or truckload has been subjected to a sterilization process. Such evidence can become a part of the batch record to confirm that sterilization was accomplished.

Further details concerning methods of sterilization and their application can be found in Chapter 25 (Sterilization Processes and Sterility Assurance). In addition, the USP provides suggestions concerning the sterilization of injections and related materials.

FREEZE-DRYING (LYOPHILIZATION)

Many parenteral drugs, particularly biopharmaceuticals, are too unstable in solution to be available as ready-to-use liquid dosage forms. Such drugs can still be filled as solutions, placed in a chamber, where the combined effects of freezing and drying under low pressure will remove the solvent and residual moisture from the solute components, resulting in a dry powder that has sufficient long term stability. The process of freeze-drying has taken on greater prominence in the parenteral industry, due to the advent of recombinant DNA technology. Proteins and peptides must be freeze-dried for clinical and commercial use. There are other technologies available to produce sterile dry powder drug products besides freeze-drying, such as sterilization and powder filling. However, freeze-drying is the most common unit process for manufacturing drug products too unstable to be marketed as solutions.

The term ‘lyophilization’ describes a process to produce a product that ‘loves the dry state.’ However, this term does not include the freezing process. Therefore, although lyophilization and freeze-drying are used interchangeably, freeze-drying is a more descriptive term. Equipment used to freeze-dry products are called freeze-dryers or lyophilizers.

Table 26-7 lists the advantages, features, and disadvantages of freeze-drying.

Freeze-drying, essentially, consists of:

- Freezing stage— Freezing the product solution at a temperature below its eutectic (crystalline) or glass transition temperature.
- Primary drying stage— Removing the solvent (ice) from the product, by evacuating the chamber, usually below 0.1 torr (100 μm Hg), and subliming the ice onto a cold, condensing surface at a temperature below that of the product, the condensing surface being within the chamber or in a connecting chamber. During primary drying, the temperature of the product must remain slightly below its critical temperature, called ‘collapse temperature.’ Collapse temperature is best measured by visual observation using a freeze-dry microscope that simulates the freeze-drying process. Collapse temperature is similar to the eutectic or glass transition temperature of the product.
- Secondary drying stage— Removing bound water from critical(s) to a level that assures long term stability of the product. This is accomplished by introducing heat to the product under controlled conditions, thereby providing additional energy to the product to remove adsorbed water. The temperature for secondary drying should be as high as possible, without causing any chemical degradation of the active ingredient. For small molecules,

Sample chapter from Remington: Essentials of Pharmacuetics
Control Computer
der graded control by electric resistance coils or by circulating refrigerant from the large compressor.
the condenser surface having been chilled previously by circulating refrigerant (usually silicone or glycol). Heat transfer proceeds from the shelf into the product vial and mass transfer (ice) proceeds from the product vial by sublimation through the chamber and onto the condenser. The process continues, until the product is dry (usually 1% or less moisture, except for some proteins that require a minimum amount of water for conformational stability), leaving a sponge-like matrix of the solids originally present in the product, the input of heat being controlled so as not to degrade the product.

For most pharmaceuticals and biologicals, the liquid product is sterilized by filtration before being filled into the dosage container aseptically. The containers must remain open during the drying process to allow water vapor to escape; therefore, they must be protected from contamination during transfer from the filling area to the freeze-drying chamber, while in the freeze-drying chamber and at the end of the drying process until sealed. Automated loading and unloading of product to and from the freeze-dryer shelves is now state-of-the-art, where partially open vials are always under the auspices of Class 100 air and human intervention is eliminated.

Freeze-dryers are equipped with hydraulic or pneumatic internal-stoppering devices designed to push slotted rubber closures into the vials to be sealed while the chamber is still evacuated, the closures having been partially inserted immediately after filling, so the slots were open to the outside. If internal stoppering is not available or containers, such as ampoules, are used, filtered dry air or nitrogen should be introduced into the chamber at the end of the process to establish atmospheric pressure.

Table 26-8 provides some guidance on a typical formulation approach and initial cycle chosen to freeze-dry a typical product.

<table>
<thead>
<tr>
<th>Table 26-7. Advantages and Disadvantages of Freeze-Drying and Desirable Characteristics of the Finished Freeze-Dried Dosage Form</th>
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</thead>
<tbody>
<tr>
<td><strong>Advantages of Freeze-Dried Products</strong></td>
</tr>
<tr>
<td>1. Product is stored in dry state—few stability problems</td>
</tr>
<tr>
<td>2. Product is dried without elevated temperatures</td>
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<tr>
<td>3. Good for oxygen and/or air-sensitive drugs</td>
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<tr>
<td>4. Rapid reconstitution time</td>
</tr>
<tr>
<td>5. Constituents of the dried material remain homogenously dispersed</td>
</tr>
<tr>
<td>6. Product is process in the liquid form</td>
</tr>
<tr>
<td>7. Sterility of product can be achieved and maintained</td>
</tr>
<tr>
<td><strong>Disadvantages of Freeze-Dried Products</strong></td>
</tr>
<tr>
<td>1. Volatile compounds may be removed by high vacuum</td>
</tr>
<tr>
<td>2. Single most expensive unit operation</td>
</tr>
<tr>
<td>3. Stability problems associated with individual drugs</td>
</tr>
<tr>
<td>4. Some issues associated with sterilization and sterility assurance of the dryer chamber and aseptic loading of vials into the chamber</td>
</tr>
<tr>
<td><strong>Desired Characteristics of Freeze-Dried Products</strong></td>
</tr>
<tr>
<td>• Intact cake</td>
</tr>
<tr>
<td>• Sufficient strength</td>
</tr>
<tr>
<td>• Uniform color</td>
</tr>
<tr>
<td>• Sufficiently dry</td>
</tr>
<tr>
<td>• Sufficiently porous</td>
</tr>
<tr>
<td>• Sterile</td>
</tr>
<tr>
<td>• Free of pyrogens</td>
</tr>
<tr>
<td>• Free of particulates</td>
</tr>
<tr>
<td>• Chemically stable</td>
</tr>
</tbody>
</table>

The highest secondary drying temperature used is 40°C, whereas for proteins it is no more than 30°C.

A small-scale lyophilization system and its functional components is shown in Figure 26-28. The product may be frozen on the shelf in the chamber by circulating refrigerant (usually silicone) from the compressor through pipes within the shelf. After freezing is complete, which may require several hours, the chamber and condenser are evacuated by the vacuum pump, the condenser surface having been chilled previously by circulating refrigerant from the large compressor.

Heat then is introduced from the shelf to the product under graded control by electric resistance coils or by circulating...
- Rate of freezing
- Set point temperatures during all three phases
- Need for annealing
- Pressure during primary drying
- Pressure during secondary drying
- Stopper seating conditions (e.g., vacuum or gas)

Optimize formulation and process based on stability information during and after freeze-drying and after storage in dry state

- Use a sample thief attachment for laboratory dryers to remove samples during the freeze-dry cycle in order to measure moisture, potency, or other parameters. Provides information for final selection of type and amount of stabilizer(s), if needed, and the cycle parameters necessary to provide an acceptable final moisture level in product

Typical freeze-dry cycle components

| Buffers: | Phosphate, citrate, acetate |
| Stabilizers: | Sucrose, trehalose, glycerine |
| Bulking agents: | Mannitol, lactose |
| Collapse temperature modifiers: | Polymers, sugars |

Typical freeze-dry cycle (without knowing where to start)

- Freezing phase
  - After loading, cool to 5°C
  - Decrease shelf temperature to -40°C
  - Hold for 2 hours
- Primary drying phase
  - Must know collapse temperature (Tc)
  - Set shelf temperature approximately 20°C above Tc but making sure product temperature is 5°C below Tc
  - Maintain chamber pressure at 10% to 30% of vapor pressure of ice at the primary drying temperature (usually 100 to 200 microns)
  - Use temperature probes, pressure rise test, or dewpoint measurement to determine end of primary drying
- Secondary drying
  - Use moderate to high vacuum (typically 100 microns)
  - Adjust shelf temperature to 25°C to 30°C for proteins; 35°C to 40°C for non proteins and hold for at least 4 hours
  - Adjust shelf temperature to 25°C or 5°C prior to stoppering, neutralizing and unloading

**Factors Affecting the Process Rate**

From the diagram in Figure 26-29, it can be seen that the direction of heat and mass transfer causes the top of the product to dry first with drying proceeding downward to the bottom of the vial. Therefore, as drying proceeds, there exists a three-component or layer system in each vial—the upper dry product, the middle sublimation front, and the lower frozen liquid product. As the dried layer increases, it becomes a greater barrier or the source of greatest resistance to the transfer of mass out of the vials. This points out the importance of vial dimensions and volume of product per vial on the efficiency of the freeze-drying process. If large volumes of solution must be processed, the surface area relative to the depth may be increased, utilizing larger vials or by using such devices as freezing the container in a slanted position to increase the surface area.

The actual driving force for the process is the vapor pressure differential between the vapor at the surface where drying of the product is occurring (the drying boundary) and at the surface of the ice on the condenser. The latter is determined by the temperature of the condenser, as modified by the insulating effect of the accumulated ice. The former is determined by a number of factors, including:

1. The rate of heat conduction through the container and the frozen material, both relatively poor thermal conductors, to the drying boundary, while maintaining all of the product below its eutectic temperature.
2. The impeding effect of the increasing depth of dried, porous product above the drying boundary.
3. The temperature and heat capacity of the shelf itself.

The passageways between the product surface and the condenser surface must be wide open and direct for effective operation. The condensing surfaces in large freeze-dryers may be in the same chamber as the product or located in a separate chamber connected by a duct to the drying chamber. Evacuation of the system is necessary to reduce the impeding effect that collisions with air molecules would have on the passage of water molecules. However, the residual pressure in the system must be greater than the vapor pressure of the ice on the condenser, or the ice will be vaporized and pulled into the pump, an event detrimental to most pumps.

The amount of solids in the product, the ice crystal size, and their thermal conductance affect the rate of drying. The more solids present, the more impediment will be provided to the escape of the water vapor. The degree of supercooling (i.e., how much lower the product temperature goes below its equilibrium

![Figure 26-29](sample-chapter.png)

*Figure 26-29.* Schematic of heat and mass transfer in the freeze dryer.
freezing point before ice crystals first form) and the rate of ice crystallization define the freezing process and efficiency of primary drying. The larger the size of ice crystals formed, usually as a result of slow freezing, the larger the pore sizes are when the ice sublimes and, consequently, the faster the rate of drying. A high degree of supercooling produces a large number of small ice crystals, a small pore size when the ice sublimes in the dried layer, and a greater resistance to water vapor transport during primary drying. The poorer the thermal conducting properties of the solids in the product, the slower the rate of heat transfer through the frozen material to the drying boundary.

The rate of drying is slow, most often requiring 24 hours or longer for completion. The actual time required, the rate of heat input, and the product temperatures used must be determined for each product and then reproduced carefully with successive processes.

FACTORS AFFECTING FORMULATION

The active constituent of many pharmaceutical products is present in such a small quantity that, if freeze-dried alone, its presence would be hard to detect visually. In fact, the solids content of the original product, ideally, should be between 5% and 30%. Therefore, excipients are often added to increase the amount of solids. Such excipients are called ‘bulking agents,’ the most commonly used bulking agent in freeze-dried formulations is mannitol. However, most freeze-dried formulations must contain other excipients, due to the need to buffer the product and/or to protect the active ingredient from the adverse effects of freezing and/or drying. Thus, buffering agents, such as sodium or potassium phosphate, sodium acetate, and sodium citrate, are commonly used in freeze-dried formulations. Sucrose, trehalose, dextran, and amino acids, such as glycine, are commonly used lyoprotectants.

Each of these substances contributes to the appearance characteristics of the plug, such as whether dull and spongy or sparkling and crystalline, firm or friable, expanded or shrunk, and uniform or striated. Therefore, the formulation of a product to be freeze-dried must include consideration not only of the nature and stability characteristics required during the liquid state, both freshly prepared and when reconstituted before use, but also the characteristics desired in the dried plug.

MODIFICATIONS IN THE PROCESS AND EQUIPMENT

In some instances, a product may be frozen in a bulk container or in trays, rather than in the final container, and then handled as a bulk solid. Such a state requires a continuation of aseptic processing conditions as long as the product is exposed to the environment.

When large quantities of material are processed, it may be desirable to use ejection pumps in the equipment system. These draw the vapor into the pump and eject it to the outside, thereby eliminating the need for a condensing surface. Such pumps are expensive and usually practical only in large installations.

Available freeze-dryers range in size from small laboratory units (Fig. 26-28), with shelf surface areas of approximately 5 square feet to large industrial models with shelf surface areas of several hundred square feet. Their selection requires consideration of such factors as:

- Tray area required,
- Volume of water to be removed,
- How the chamber will be sterilized,
- Whether internal stoppering is required,
- Whether separate freezers will be used for initial freezing and condensation of the product, and
- Degree of automatic operation desired.

Other factors involved in the selection and use of equipment are considered in the literature.35

Freeze-drying is now used for research in the preservation of human tissue and is finding increasing application in the food industry. Most biopharmaceuticals require lyophilization to stabilize their protein content effectively. Therefore, many newer developments in the lyophilization process focus on the requirements of this new class of drug products.

QUALITY ASSURANCE AND CONTROL

The importance of undertaking every possible means to ensure the quality of the finished product cannot be overemphasized. Every component and step of the manufacturing process must be subjected to intense scrutiny to be confident quality is attained in the finished product. The responsibility for achieving this quality is divided appropriately in concept and practice into Quality Assurance (QA) and Quality Control (QC). QA relates to the studies made and the plans developed for ensuring quality of a product prospectively, with a final confirmation of achievement. QC embodies the carrying out of these plans during production and includes all of the tests and evaluations performed to ensure quality exists in a specific lot of product.

The principles for achieving quality are basically the same for the manufacture of any pharmaceutical. These are discussed in Chapter 3 (Quality Assurance and Control) During the discussion of preparation of injections in this chapter, mention was made of numerous quality requirements for components and manufacturing processes. Here, only selected tests characteristically required before a finished parenteral product is released are discussed briefly, including sterility, pyrogen, and particulate tests.

STERILITY TEST

All lots of injectables in their final containers must be tested for sterility, except products that are allowed to apply parametric release (i.e., terminally sterilized by a well-defined, fully validated sterilization process, has a sterility assurance level sufficient to omit the sterility test for release). The USP prescribes the requirements for this test for official injections. The FDA uses these requirements as a guide for testing official sterile products. The primary official test is performed by means of filtration, but direct transfer is used if membrane filtration is unsuitable. To give greater assurance that viable micro-organisms will grow, if present, the USP requires that all lots of culture media be tested for their growth-promotion capabilities. However, it must be recognized that the reliability of both test methods has the inherent limitations typical of microbial recovery tests. Therefore, it should be noted that this test is not intended as a thorough evaluative test for a product subjected to a sterilization method of unknown effectiveness. It is intended as a check test on the probability that a previously validated sterilization procedure has been repeated or to give assurance of its continued effectiveness. A discussion of sterility testing is given in Chapter 25 (Sterilization Processes and Sterility Assurance).

In the event of a sterility-test failure, the immediate issue concerns whether the growth observed came from viable micro-organisms in the product (true contamination) or from adventitious contamination during the testing (a false positive). The USP does not permit a retest, unless specific evidence is discovered to suggest contamination occurred during the test. Therefore, a thorough investigation must be launched to support the justification for performing the retest and assessing the validity of the retest results relative to release of the lot of product.

It should be noted that a ‘lot,’ with respect to sterility testing, is a group of product containers that has been subjected to the same sterilization procedure. For containers of a product that have been sterilized by autoclaving, for example, a lot would constitute those processed in a particular sterilizer cycle. For an aseptic filling operation, a lot would constitute all of those product containers filled during a period in which there was no change in the filling assembly or equipment and which is no longer than one working day or shift.
As stated previously, isolator technology has been applied to significantly reduce the incidence of false positives in the conductance of the sterility test. Figure 26-10 shows an example of a sterility testing isolator. Validation of isolator systems for sterility testing is described in USP <1208>.

## PYROGEN TEST

The USP evaluates the presence of pyrogens in parenteral preparations by a qualitative fever response test in rabbits, the Pyrogen Test (Section <151>), and by the Bacterial Endotoxins Test (Section <85>). These two USP tests are described in Chapter 25 (Sterilization Processes and Sterility Assurance). Rabbits are used as test animals in Section <151>, because they show a physiological response to pyrogenic substances similar to that of man. Although a minimum pyrogenic dose (MPD), the amount just sufficient to cause a positive USP Pyrogen Test response, may produce uncertain test results, a content equal to a few times the MPD will leave no uncertainty. Therefore, the test is valid and has continued in use, since introduced by Seibert in 1923. It should be understood that not all injections may be subjected to the rabbit test, since the medicinal agent may have a physiological effect on the test animal such that any fever response would be masked.

The Bacterial Endotoxins Test (BET) is an in vitro test based on the formation of a gel or the development of color in the presence of bacterial endotoxins and the lysate of the amebocytes of the horseshoe crab (*Limulus polyphemus*). The Limulus Ameboocyte Lysate (LAL) test, as it also is called, is a biochemical test performed in a test tube and is simpler, more rapid, and of greater sensitivity than the rabbit test. Figure 26-30 shows an example of a positive endotoxin test result in a test tube. Although it detects only the endotoxic pyrogens of gram-negative bacteria, these are the most prominent environmental microbial contaminants likely to invade sterile products. The test has been automated and can determine the quantitative amount of endotoxin in a sample. This test has enabled endotoxin limits to be established on finished products and bulk drug substances and excipients.

To provide standardization for the test, the USP has established a reference standard endotoxin (RSE) against which lots of the lysate is standardized. Thus, the sensitivity of the lysate is given in terms of endotoxin units (EU). Most USP injections have now been given limits in terms of EU’s (e.g., Bacteriostatic Sodium Chloride Injection, 1.0 EU/mL), thus, indicating an increasing priority for the BET in testing for the presence of endotoxin in parenteral products and in medical devices.

### PARTICULATE MATTER EVALUATION

Particulate matter in parenteral solutions has been recognized as unacceptable, since the user could be expected to conclude that the presence of visible dirt would suggest that the product is of inferior quality. Today, it is recognized that the presence of particles in solution, particularly if injected intravenously, can be harmful. Although data defining the extent of risk and the effects produced are still limited, it has been shown that particles of lint, rubber, insoluble chemicals, and other foreign matter can produce emboli in the vital organs of animals and man. Further, it has been shown that the development of infusion phlebitis may be related to the presence of particulate matter in intravenous fluids.

The particle size of particular concern has not been clearly delineated, but it has been suggested that, since erythrocytes have a diameter of approximately 4.5 μm, particles of more than 5 μm should be the basis for evaluation. This is a considerably smaller particle than can be seen with the unaided eye; approximately 50 μm is the lower limit, unless the Tyndall effect is used, whereby particles as small as 10 μm can be seen by the light scattered from them.

The USP specifies that good manufacturing practice requires each final container of an injection be subjected individually to a visual inspection and containers in which visible particles can be seen should be discarded. This 100% inspection of a lot of product is designed to prevent the distribution and use of parenterals that contain particulate matter. Therefore, all of the product units from a production line are currently being inspected individually, by human inspectors, under a good light, baffled against reflection into the eye, and against a black-and-white background. This inspection is subject to the limitation of the size of particles that can be seen, the variation of visual acuity from inspector to inspector, their emotional state, eye strain, fatigue, and other personal factors that will affect what is seen. However, it does provide a means for eliminating the few units that normally contain visible particles. Automated inspection machines are increasingly being used today.

The assessment of the level of particulate matter below the visible size of about 50 μm has become an increasingly used QC indicator of process cleanliness in the manufacture of injections. The tests used, however, are destructive of container units. Therefore, they are performed on appropriately selected samples of products. Further, all of these methods require very stringent, ultraclean preparation techniques to ensure accuracy in the counting and sizing of particles only in the product, rather than those that may have been introduced inadvertently during the sample preparation or the testing procedure.

The USP has identified two test methods in <788>, *Particulate Matter in Injections*. All LVIs for single-dose infusion and those SVIs for which the monograph specifies a limit (primarily those commonly added to infusion solutions) are subject to the specified limits given in Table 26-9. The first test used is the light obscuration test, which uses an electronic instrument designed to count and measure the size of particles by means of a shadow cast by the particle as it passes through a high-intensity light beam. If the injection formulation is not a clear, colorless solution (e.g., an emulsion) or it exceeds the limits specified for the light obscuration test, it is to be subjected to the microscopic count test. The latter method consists of filtering a measured sample of solution through a membrane filter under ultraclean conditions and then counting the particles on the surface of the filter, using a microscope and oblique light at 100x magnification. The time requirements for performing the latter test are very long. These standards are readily met in the United States today by the manufacturers of LVIs and the specified SVIs.

Whether or not these standards are realistic, toxicologically has not been established; rather, the objective of the compendium is to establish specification limits that encourage the preparation of clean parenteral solutions, particularly those to be given intravenously.
It also should be realized that administration sets and the techniques used for preparing and administering intravenous infusion fluids may introduce substantial amounts of particulate matter into an otherwise clean solution. Therefore, the pharmaceutical manufacturer, the administration set manufacturer, the pharmacist, the nurse, and the physician must share responsibility for making sure the patient receives a clean intravenous injection.

### CONTAINER/CLOSURE INTegrity TEST

Ampoules that have been sealed by fusion must be subjected to a test to determine whether or not a passageway remains to the outside; if so, all or a part of the contents may leak to the outside and spoil the package, or micro-organisms or other contaminants may enter. Changes in temperature during storage cause expansion and contraction of the ampoule and contents, and will accentuate interchange, if a passageway exists, even if microscopic in size.

This test is usually performed by producing a negative pressure within an incompletely sealed ampoule, while the ampoule is submerged entirely in a deeply colored dye solution. Most often, approximately 1% methylene blue solution is employed. After carefully rinsing the dye solution from the outside, color from the dye will be visible within a leaker. Leakers, of course, are discarded.

Vials and bottles are not subjected to such a leaker test, because the sealing material (rubber stopper) is not rigid. Therefore, results from such a test would be meaningless. However, assurance of container-closure sealing integrity should be an integral part of product development, by developing specifications for the fit of the closure in the neck of the container, the physical characteristics of the closure, the need for lubrication of the closure, and the capping pressure.

### SAFETY TEST

The National Institutes of Health requires, of most biological products, routine safety testing in animals. Under the Kefauver-Harris Amendments to the Federal Food, Drug, and Cosmetic Act, most pharmaceutical preparations are now required to be tested for safety. Because it is entirely possible for a parenteral product to pass the routine sterility test, pyrogen test, and chemical analyses, and still cause unfavorable reactions when injected, a safety test in animals is essential, particularly for biological products, to provide additional assurance that the product does not have unexpected toxic properties.

### PACKAGING AND LABELING

A full discussion of the packaging of parenteral preparations is beyond the scope of this text. It is essential, of course, that the packaging provide ample protection for the product against physical damage from shipping, handling, and storage, as well as protecting light-sensitive materials from ultraviolet radiation.

### PACKAGING

The USP includes certain requirements for the packaging and storage of injections:

1. The volume of injection in single-dose containers is defined as that which is specified for parenteral administration at one time and is limited to a volume of 1 L.
2. Parenterals intended for intraspinal, intracisternal, or peridural administration are packaged only in single-dose containers.
3. Unless an individual monograph specifies otherwise, no multiple-dose container shall contain a volume of injection more than sufficient to permit the withdrawal and administration of 30 mL.
4. Injections packaged for use as irrigation solutions or for hemofiltration or dialysis or for parenteral nutrition are exempt from the foregoing requirements relating to packaging. Containers for injections packaged for use as hemofiltration or irrigation solutions may be designed to empty rapidly and may contain a volume in excess of 1 L.
5. Injections intended for veterinary use are exempt from the packaging and storage requirements concerning the limitation to single-dose containers and to volume of multiple-dose containers.

### LABELING

The labeling of an injection must provide the physician or other user with all information needed to ensure the safe and proper use of the product. Since all of this information cannot be placed on the immediate container and be legible, it may be provided on accompanying printed matter.

A restatement of the labeling definitions and requirements of the USP for injections is as follows:

The term ‘labeling’ designates all labels and other written, printed, or graphic matter upon an immediate container or upon, or in, any package or wrapper in which it is enclosed, with the exception of the outer shipping container. The term ‘label’ designates part of the labeling upon the immediate container.

The label states the name of the preparation, the percentage content of drug of a liquid preparation, the amount of active ingredient of a dry preparation, the volume of liquid to be added to prepare an injection or suspension from a dry preparation, the route of administration, a statement of storage conditions, and an expiration date. The label must state the name of the vehicle and the proportions of each constituent, if it is a mixture, and the names and proportions of all substances added to increase stability or usefulness.

Also, the label must indicate the name of the manufacturer or distributor and carry an identifying lot number. The lot number is capable of providing access to the complete manufacturing history of the specific package, including each
single manufacturing step. The container label is so arranged that a sufficient area of the container remains uncovered for its full length or circumference to permit inspection of the contents.

Preparations labeled for use as dialysis, hemofiltration, or irrigation solutions must meet the requirements for injections, other than those relating to volume, and must also bear on the label statements that they are not intended for intravenous injection. Injections intended for veterinary use are so labeled.

REFERENCES


BIBLIOGRAPHY


