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CHAPTER 87

Parenteral Preparations

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The distinctive characteristics of parenteral (Gk, *para enteron*, beside the intestine) dosage forms of drugs will be discussed in this chapter. These dosage forms differ from all other drug dosage forms because of the unique requirements imposed because they are injected directly into body tissue through the primary protective system of the human body, the skin and mucous membranes. Therefore, 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 good manufacturing practices (GMPs) in the manufacture of parenteral dosage forms and upon pharmacists to practice good aseptic practices (GAPs) in dispensing them for administration to patients.

Many of the newer drugs, particularly these derived from the new developments in biotechnology, can only be given parenterally because they are inactivated in the gastrointestinal tract, when given by mouth. Further, the potency and specificity of many of these drugs requires strict control of their administration to the patient. A parenteral route of administration meets both of these critical requirements.

This chapter will begin with a brief review of the historical events contributing to the development of this distinctive dosage form. Consideration will then be given to some of the distinguishing characteristics of these dosage forms and how they are administered to patients. The majority of the remainder of the chapter will discuss the various factors required for the preparation of a pure, safe and effective parenteral product.

History[1]

One of the most significant events in the beginnings of parenteral therapy was the first recorded injection of drugs into the veins of living animals, in about 1657, by the architect Sir Christopher Wren. From such a very crude beginning, the technique for intravenous injection and knowledge of the implications therefore developed slowly during the next century and a half. In 1855 Dr Alexander Wood of Edinburgh described what was probably the first subcutaneous injection of drugs for therapeutic purposes using a true hypodermic syringe.

The latter half of the 19th century brought increasing concern for safety in the administration of parenteral solutions, largely because of the work of Robert Koch and Luis Pasteur. While Charles Chamberland was developing both hot-air and steam sterilization techniques and the first bacteria-retaining filter (made of unglazed porcelain), Stanislaus Limousin was developing a suitable container, the all-glass ampul. In the middle 1920s Dr Florence Seibert provided proof that the disturbing chills and fever which often followed the intravenous injection of drugs was caused by potent products of microbial growth, pyrogens, which could be eliminated from water by distillation and from glassware by heating at elevated temperatures.

Of the technical developments that have contributed to the high quality standards currently achievable in the preparation of parenteral dosage forms, the two that have probably contributed most are the development of HEPA-filtered laminar airflow and the development of membrane microfiltration for solutions. The former made it possible to achieve ultraclean environmental conditions for processing from solutions by filtration both viable and nonviable particles of microbial size and smaller. However, many other developments in recent years have produced an impressive advance in the technology associated with the safe and reliable preparation of parenteral dosage forms. The following list identifies a few of the events which have contributed to that development.

1926—Parenterals were accepted for inclusion in the fifth edition of the *National Formulary*.

1933—The practical application of freeze-drying to clinical materials was accomplished by a team of scientists at the University of Pennsylvania.

1938—The Food, Drug and Cosmetic Act was passed by Congress, establishing the Food and Drug Administration (FDA).

1944—The sterilant ethylene oxide was discovered.

1946—The Parenteral Drug Association was organized.

1961—The concept of laminar airflow was developed by WJ Whitfield.

1962—The FDA was authorized by Congress to establish current good manufacturing practice (CGMP or GMP) regulations.

1965—Total parenteral nutrition (TPN) was developed by SJ Dudrick.

1972—The Limulus Amebocyte Lysate test for pyrogens in parenteral products was developed by JF Cooper.

1974—The concept of validation of processes used in the manufacture of parenteral products was introduced by the FDA.

1977—The principles for clean-in-place (CIP) and steam-in-place (SIP) were introduced.

Early 1980s—Home Health Care emerged as an alternative for patients whose health status permitted release from a hospital to care in the home environment.

1982—Insulin, derived through the new discipline of biotechnology, ushered in the drug class of polypeptides with their inherent stability challenges for parenteral dosage-form development.

1987—Parametric release was accepted by the FDA for selected products terminally sterilized by a validated heat process.

The FDA published *Guideline on Sterile Products Produced by Aseptic Processing*, one of several nonregulatory publications to help industry know what the FDA considers to be acceptable.

Late 1980s—The development of computer capabilities has led to the automation of many process operations and to a revolution in documentation and recordkeeping.

1991—The FDA proposed requiring manufacturers to use a terminal sterilization process when preparing a sterile drug product unless such a process adversely affects the drug product.

Administration

Injections may be classified in six general categories:

1. Solutions ready for injection.
2. Dry, soluble products ready to be combined with a solvent just prior to use.
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.

These injections may be administered by such routes as intravenous, subcutaneous, intradermal, intramuscular, intra-

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articular and intrathecal. The nature of the product will determine the particular route of administration that may be employed. Conversely, the desired route of administration will place requirements on the formulation. For example, suspensions would not be administered directly into the blood stream because of the danger of insoluble particles blocking capillaries. Solutions to be administered subcutaneously require strict attention to tonicity adjustment, otherwise irritation of the plentiful supply of nerve endings in this anatomical area would give rise to pronounced pain. Injections intended for intraocular, intraspinal, intracisternal and intrathecal administration require the highest purity standards because of the sensitivity of tissues encountered to irritant and toxic substances.

When compared with other dosage forms, injections possess select advantages. If immediate physiological action is needed from a drug, it usually can be provided by the intravenous injection 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 normally is administered by a professionally trained person, it confidently may be expected that the dose was actually and accurately administered. Drugs can be administered parenterally when they cannot be given orally because of the unconscious or uncooperative state of the patient, or because of 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 factor 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 impossible. One other disadvantage is that daily or frequent administration poses difficulties, either for the patient to visit a professionally trained person or to learn to inject oneself. However, the advent of home health care as an alternative to extended institutional care has mandated the development of programs for training lay persons to administer these dosage forms.

Parenteral Combinations

During the administration of large-volume parenterals (LVPs), such as 1000-mL of 0.9% sodium chloride solution, it is common practice for a physician to order the addition of a small-volume therapeutic parenteral (SVP), such as an antibiotic, to avoid the discomfort for the patient of a separate injection. While the pharmacist is the most qualified health professional to be responsible to prepare such combinations, as is clearly stated in the Hospital Accreditation Manual of the Joint Commission on Accreditation of Healthcare Organizations,[2] interactions among the combined products can be troublesome even for the pharmacist. In fact, incompatibilities can occur and cause inactivation of one or more ingredients or other undesired reactions. 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 situation even for the pharmacist. To aid in making decisions concerning potential problems, a valuable compilation of relevant data has been assembled by Trissel,[3] and is regularly updated. 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 88.

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 constituents aids substantially in understanding and predicting potential incompatibilities. Kinetic studies of reaction rates may be used to describe or predict the extent of degradation. Ultimately, a thorough study should be undertaken of each therapeutic agent in combination with other drugs and IV fluids, not only of generic but 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 the safety of the combination. However, such an ideal situation may not exist. Nevertheless, it is the responsibility 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 to 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 safety for the patient. Therefore, whether they are prepared from commercially available sterile components, as is usually the case in hospital pharmacies and similar sites, or from nonsterile ingredients in a manufacturing mode, as is the case in the pharmaceutical industry, the persons responsible for their preparation must apply their skills intelligently and diligently. Further, the possession and application of high moral and professional ethics on the part of the persons responsible is the ingredient most vital to achieving the desired quality in the products prepared.

The preparation of parenteral products from sterile components in pharmacies of hospitals and similar sites is discussed further in Chapter 88. In this chapter emphasis will be placed on the preparation of parenteral products from non-sterile components in the highly technologically advanced plants of the pharmaceutical industry, using GMP principles. In the pursuit of GMP, consideration should be given to:

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

The pursuit of GMP is an ongoing effort which must flex with new technological developments and new understanding of existing principles. Because of the extreme importance of quality in health care of the public, the US Congress has given the responsiblity 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 oversite of the FDA and, with respect to manufacturing practices, to the application of the CGMPs.[4] These regulations are discussed more fully in Chapter 110.

In concert with the pursuit of GMPs, 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. Further, outstanding innovative development in drug-delivery

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systems is occurring. These factors have been additive in providing the public with outstanding parenteral dosage forms of drugs at this time in history.

General Manufacturing Process

The preparation of a parenteral product may be considered to encompass four general areas as follows:

1. Procurement and selection o the components and containers.
2. Production facilities and procedures.
3. Control of quality.
4. Packaging and labeling.

These components of the product to be procured include vehicles, solutes, containers and closures. The steps constituting production include maintaining facilities and equipment, preparing and controlling the environment, cleaning the containers and equipment, preparing the product, filtering the solution, filling containers with the product, sealing the containers and sterilizing the product. The control of quality includes the evaluation of the components, qualification of equipment, validation of processes, determination that the production has been executed within prescribed requirements and performance of necessary evaluative tests on the finished product. The final area of packaging and labeling includes all steps necessary to identify the finished product and enclose it in such manner that it is safely and properly prepared for sale and delivery to the user. The remainder of this chapter will be organized in accord with these four general areas, with emphasis on the first two areas.

Components and Containers

Establishing specifications to ensure the quality of each of the components of an injection is essential. These specifications will be coordinated with the requirements of the specific formulation and will not necessarily be identical for a particular component if used in several different formulations. For example, particle-size control may be necessary for powders used in formulating a suspension but be relatively unimportant for preparing a solution.

The most stringent chemical-purity requirements normally will be encountered with aqueous solutions, particularly if the product is to be sterilized at an elevated temperature where reaction rates will be accelerated greatly. Modification of aqueous vehicles to include a glycol, for example, usually will reduce reaction rates. 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. Assessment and selection of containers and closures is a necessary part of product formulation to ensure that the product retains its purity, potency and quality during the intimate contact with the container throughout its shelf-life. Administration devices 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.

Vehicles

Since most liquid injections are quite dilute, the component present in the highest proportion is the vehicle. A vehicle normally has no therapeutic activity and is nontoxic. However, it is of great importance in the formulation since it presents to body tissues the form of the active constituent for absorption. Absorption normally occurs most rapidly and completely when a drug is presented as an aqueous solution. Modification of the vehicle with water-miscible liquids or substitution with water-immiscible liquids normally decreases the rate of absorption. Absorption from a suspension may be affected by such factors as the viscosity of the vehicle, its capacity for wetting the solid particles, the solubility equilibrium produced by the vehicle and the distribution coefficient between the vehicle and aqueous body systems.

The vehicle of greatest importance for parenteral products is water. Water of suitable quality for compounding and rinsing product contact surfaces may be prepared either by distillation or by reverse osmosis, to meet USP specifications for Water for Injection (WFI). Only by these two methods is it possible to separate adequately various liquid, gas and solid contaminating substances from water. These two methods for preparation of WFI will be discussed in this Chapter. It should be noted that there is no unit operation more important and none more costly to install and operate than the one for the preparation of WFI.

Preparation of Water for Injection (WFI)

The source water can be expected to be contaminated with natural suspended mineral and organic substances, dissolved mineral salts, colloidal silicates and industrial chemicals. The degree of contamination will vary with the source and will be markedly different whether obtained from a well or from surface sources, such as a stream or lake. Therefore, this water normally is not of sufficient purity to prepare WFI directly. Hence, the source water usually must be pretreated by one or a combination of the following treatments: chemical softening, filtration, deionization, carbon adsorption or reverse osmosis purification. Space does not permit discussion of these processes here, but the interested reader is referred elsewhere for this information.[5,6]

In general, 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, a means for eliminating volatile impurities 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 markedly will affect the quality of distillate obtained from a still. Those required for producing high-purity water, such as WFI, must be considerably more stringent than those required for Purified Water USP. Among the factors that must be considered are:

1. The quality of the feed water will affect the quality of the distillate. 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 to remove efficiently 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.

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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, such as shown in Fig 1, are available from several suppliers (*AMSCO, Barnstead, Corning, Vaponics*).

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 Fig 2. To start, the feed water is heated 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 which compresses the vapor and raises its temperature to approximately 107°. It then flows to the steam chest where it condenses on the outer surfaces of the tubes containing the distilland; thereby the vapor is 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 (*Aqua-Chem, Barnstead, Meco*).

Multiple-Effect Stills—The multiple-effect still also is designed to conserve energy and water usage. In principle, it is simply a series of single-effect stills running at differing pressures. 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. See a schematic drawing of a multiple-effect still in Fig. 3. 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.

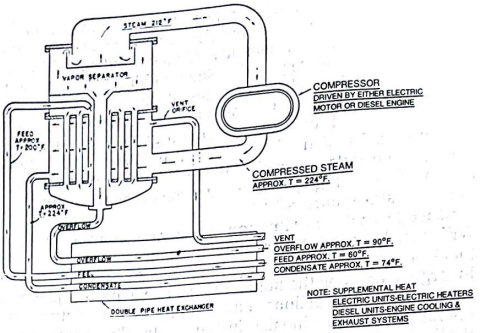


Fig 2. Vapor-compression still.

The capacity of a multiple-effect still can be increased by adding effects. The quantity of the distillate also will 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 (*AMSCO, Barnstead, Finn-Aqua, Vaponics*).

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 premeate 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 ones such as sodium chloride. Passage through two membranes in series is sometimes used to increase the efficiency of removal of these small molecules and to decrease the risk of structural failure of a membrane to remove other contaminants, such as bacteria and pyrogens. For additional information, see *Reverse Osmosis* in Chapter 37 and *Water* in Chapters 80 and 86.

Reverse osmosis systems are available in a range of production sizes. (*AMSCO, Aqua-Chem, Finn-Aqua, Meco, Millipore*, etc).

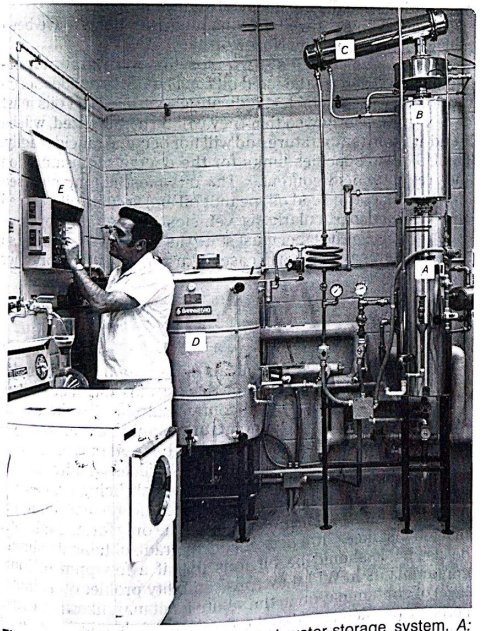


Fig 1. High-purity stilled and sealed water-storage system. *A:* evaporator; *B:* high-purity baffle unit; *C:* condenser; *D:* storage tank with ultraviolet lamp; *E:* control panel (courtesy, Ciba-Geigy).

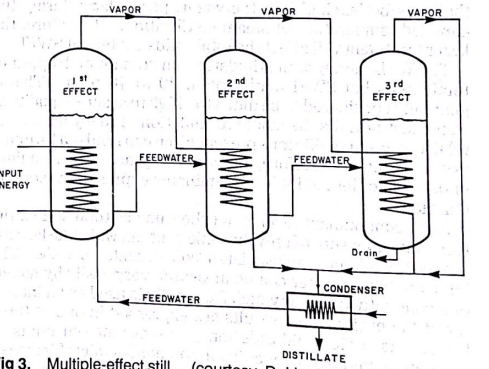


Fig 3. Multiple-effect still. (courtesy, Dekker); taken from Avis KE, Lieberman HA, Lachman L; *Pharmaceutical Dosage Forms: Parenteral Medications*, vol 2, 2nd ed, Dekker, New York, 1993.

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Whichever system is used for the preparation of WFI, validation is required to be sure that the system consistently and reliably will produce 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 determine the ultimate quality of the WFI. Because of space limitations here, more details concerning the design, operation and validation of these highly important systems may be found in other literature sources.[5,6]

Storage and Distribution—The rate of production of WFI usually 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 to occur. Normally, this temperature is a constant 80°.

The USP also permits the WFI to be stored at room temperature, but for a maximum of 24 hours. Under such conditions the WFI usually is collected as a batch for a particular use with any unused water being 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 usually are 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°, heat exchangers must be installed to reduce the temperature at the point of use. Bacterial retentive filters should not be installed in such systems because of the risk of bacterial buildup on the filters and the consequential release of pyrogenic substances.

Purity—The USP monographs provide standards of purity for WFI and Sterile Water for Injection (SWFI). A few of these standards require comment.

SWFI must meet the requirements of the USP Sterility Test, but WFI need not since it is to be used in a product which will be sterilized. Both must contain not more than 0.25 Endotoxin units per mL.

The limits for total solids varies in the two monographs. The larger the surface area of the glass container per unit volume of water, the greater the amount of glass constituents that may be leached into the water, particularly during the elevated temperature of steam sterilization. Therefore, the latter treatment will affect the total solids content of SWFI.

The WFL monograph stipulates a maximum of 10 ppm of total solids but SWFI may contain 20 to 40 ppm. This is generally considered to be much too high to assure a quality of water that permits the stable formulation of many drugs. A relatively few metallic ions present often can render a formulation unstable. Therefore, it is common practice to set a limit of 0.1 ppm or less of ionic contaminants expressed as sodium chloride.

Ionic contaminant level is not the same as total solids; the former is a measure of only the ionic content, while the latter is a measure of the undissociated constituents as well. The ionic content of water can be measured very easily by means of a conductivity meter which frequently is used as an indicator of the purity. The results are expressed in one of three terms: as sodium chloride ions, as resistance in ohms or megohms or as conductance in micromhos (or microSiemens). Ohms and mhos have a reciprocal relationship to each other, but they are related to ppm sodium chloride by an experimentally determined curve. The USP is considering introducing a conductivity requirement. To give one point of comparison, 0.1 ppm sodium chloride is equal to approximately 1.01 megohms and 0.99 micromhos. It should be mentioned that conductivity measurements give no direct indication of pyrogen content since pyrogens are undissociated organic compounds.

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 probably would 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.

Types of Vehicles

Aqueous Vehicles—Certain aqueous vehicles are recognized officially because of 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.

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 primarily to affect the solubility of certain drugs and to reduce hydrolysis. The most important solvents in this group are ethyl alcohol, polyethylene glycol and propylene glycol. Ethyl alcohol is used particularly in the preparation of solutions of cardiac glycosides and the glycols in solutions of barbiturates, certain alkaloids and certain antibiotics. Such preparations usually are given intramuscularly.

These solvents, as well as nonaqueous vehicles, have been reviewed by Spiegel and Noseworthy.[7]

Nonaqueous Vehicles—The most important group of nonaqueous vehicles are the fixed oils. The USP provides specifications for such vehicles, indicating that the fixed oils must be of vegetable origin so that they will be metabolized, will be liquid at room temperature and will not become rancid readily. The USP also specifies limits for the degree of unsaturation and free fatty acid content. The oils most commonly used are corn oil, cottonseed oil, peanut oil and sesame oil. Fixed oils are used particularly as vehicles for certain hormone preparations. The label must state the name of the vehicle so that the user may beware in case of known sensitivity or other reactions to it.

Solutes

Bulk pharmaceutical chemicals should be prepared under conditions designed to preclude the introduction of chemical, physical and microbiological contamination. To the extent possible, the system of manufacture should be a closed system, particularly if the chemical is to be used in a parenteral formulation. Further, the contact surfaces of all process equipment should be inert to the chemicals being processed. Where the chemicals are to be used in relatively small amounts, marketed commercial grades may be the only forms available. In such instances, the best chemical grade obtainable should be used. It should be obvious that if a few ppm of ionic contaminants in WFI may cause stability problems, a similar level of contamination in the solute itself may, likewise, cause stability problems. Metallic catalysis of chemical reactions is one which is encountered frequently.

Other factors to be considered with respect to the quality of solutes include the level of microbial and pyrogenic contami-

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nation, solubility characteristics as determined by the chemical or physical form of the compound and freedom from gross dirt.

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

Effect solubility, as does sodium benzoate in Caffeine and Sodium Benzoate Injection.

Provide patient comfort, as do substances added to make a solution isotonic.

Enhance the chemical stability of a solution, as do antioxidants, inert gases, chelating agents and buffers.

Protect a preparation against the growth of microorganisms. The term "preservative" sometimes is applied only to those substances which prevent the growth of microoganisms 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.

While 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 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.

Antimicrobial Agents—The USP states that antimicrobial agents in bacteriostatic or fungistatic concentrations must be added to preparations contained in multiple-dose containers. 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 concentration limits for those that are used commonly in parenteral products, as follows:

Phenylmercuric nitrate and thimerosal 0.01%.
Benzethonium chloride and benzalkonium chloride 0.01%.
Phenol or cresol 0.5%.
Chlorobutanol 0.5%.

The above limit is rarely used for phenylmercuric nitrate, most frequently being employed in a concentration of 0.002%. Methyl *p*-hydroxybenzoate 0.18% and propyl *p*-hydroxybenzoate 0.02% in combination, and benzyl alcohol 2% also are used frequently. In oleaginous preparations, no antibacterial agent commonly employed appears to be effective. However, it has been reported that hexylresorcinol 0.5% and phenylmercuric benzoate 0.1% are moderately bactericidal. A few therapeutic compounds have been shown to have antibacterial activity, thus obviating the need for added agents.

Antimicrobial agents must be studied with respect to compatibility with all other components of the formula. In addition, their activity must be evaluated in the total formula. It is not uncommon to find that a particular agent will be effective in one formulation but ineffective in another. This may be due to the effect of various components of the formula on the biological activity or availability of the compound; for example, the binding and inactivation of esters of *p*-hydroxybenzoic acid by macromolecules such as Polysorbate 80 or the reduction of phenylmercuric nitrate by sulfide residues in rubber closures. A physical reaction encountered is that bacteriostatic agents sometimes are removed from solution by rubber closures.

Single-dose containers and pharmacy bulk packs which do not contain antimicrobial agents are expected to be used promptly after opening or to be discarded. 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 can support the growth of microorganisms, such as total parenteral nutrition (TPN) solutions. It should be noted that while refrigeration slows the growth of most microorganisms, it does not prevent their growth.

Buffers are used primarily to stabilize a solution against the chemical degradation that might occur if the pH changes appreciably. Buffer systems employed should normally have as low a buffer capacity as feasible in order not to disturb significantly the body buffer systems when injected. In addition, the buffer range and effect on the activity of the product must be evaluated carefully. The acid salts most frequently employed as buffers are citrates, acetates and phosphates.

Antioxidants are required frequently to preserve products because of the ease with which many drugs are oxidized. Sodium bisulfite 0.1% is used most frequently. The use of sulfites has been reviewed by Schroeter.[8] Acetone sodium bisulfite, sodium formaldehyde sulfoxylate and thiourea also are used sometimes. The sodium salt of ethylenediaminetetraacetic acid has been found to enhance the activity of antioxidants in some cases, apparently 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, also can be used as a means to control oxidation of a sensitive drug. Process control is required to be assured that every container is deaerated adequately and uniformly.

Tonicity Agents are used in many parenteral and ophthalmic products to control the tonicity. However, not all preparations need to be isotonic. The agents most commonly used are electrolytes and mono- or disaccharides. This subject is considered much more extensively in Chapter 36.

Pyrogens (Endotoxins)

Pyrogens are products of metabolism of microorganisms. The most potent pyrogenic substances (endotoxins) are constituents of the cell wall of Gram-negative bacteria. Gram-positive bacteria and fungi also produce pyrogens but of lower potency and of different chemical nature. Endotoxins are high-molecular-weight (about 20,000 daltons) lipopolysaccharides. Studies have shown that 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, this discussion will focus 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. While 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 will be 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, leucocytic phagocytosis is believed to occur and endogenous pyrogen is produced. The endogenous pyrogen then produces the familiar physiological effects. Space will not permit further elaboration of these matters here, but the reader is referred to the work by Pearson[9] if more information is needed.

Control of Pyrogens—Pyrogens are contaminants if present in parenteral drug products, and should not be there. In general, it is impractical, if not impossible, to remove pyrogens once present without adversely affecting the drug product. Therefore, the emphasis should be on the prevention of 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 microorganisms. However, current technology generally permits the control of such contamination, and the presence of pyrogens in a finished product is

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indicative of processing under inadequately controlled clean 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 one work day.

Pyrogens can be destroyed by heating at high temperatures. The recommended procedure for depyrogenation of glassware and equipment is heating at a temperature of 250° for 45 minutes. It has been reported that 650° for 1 minute or 180° for 4 hours likewise will destroy pyrogens. The usual autoclaving cycle will not do so. Heating with strong alkali or oxidizing solutions will destroy pyrogens. It has been claimed that thorough washing with detergent will render glassware pyrogen-free if subsequently rinsed thoroughly with pyrogen-free water. Plastic containers and devices must be protected from pyrogenic contamination during manufacture and storage since known ways to destroy pyrogens will affect the plastic adversely. It has been reported that anion-exchange resins and positively charged membrane filters will remove pyrogens from water. Also, reverse osmosis will eliminate them. However, 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 also may 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. Developments in ultrafiltration now make possible pyrogen separation on a molecular weight basis and the process of tangential flow is making large-scale processing a reality.

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 microorganisms. When microorganisms 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 that they will become pyrogenic. As stated previously, although proper distillation will provide pyrogen-free water, storage conditions must be such that microorganisms 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. 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 will reduce and subsequent dry-heat treatment can render contaminated equipment suitable for use. However, all such processes must be validated to assure their effectiveness.

Solutes 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 fermentation will almost certainly be heavily pyrogenic. Therefore, all lots of solutes used to prepare parenteral products should be tested to ensure that they will not contribute unacceptable quantities of endotoxin to the finished product.

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.

Containers

Containers are an integral part of the formulation of an injection. No container is totally insoluble or does not in some way affect the liquid it contains, particularly if the liquid is aqueous. Therefore, the selection of a container for a particular injection must be based on a consideration of the composition of the container, as well as of the solution, and the treatment to which it will be subjected.

Table 1 provides a generalized comparison of the three compatibility properties—leaching, permeation and adsorption—of container materials most likely to be involved in the formulation of aqueous parenterals. Further, the integrity of the container/closure system depends upon several characteristics, including container opening finish, closure modulus, durometer and compression set, and aluminum seal application force. These considerations have been reviewed by Morton.[10]

Table 1—Comparative Compatibility Properties of Container Materials

	Leaching		Permeation		Adsorption (selective) extent[a]
	Extent[a]	Potential leachables	Extent[a]	Potential agents	
Glass					
Borosilicate	1	Alkaline earth and heavy metal oxides	0	N/A	2
Soda-lime	5	Alkaline earth and heavy metal oxides	0	N/A	2
Plastic polymers					
Polyethylene					
Low density	2	Plasticizers, antioxidants	5	Gases, water vapor, other molecules	2
High density	1	Antioxidants	3	Gases, water vapor, other molecules	2
PVC	4	HCl, especially plasticizers, antioxidants, other stabilizers	5	Gases, especially water vapor and other molecules	2
Polyolefins	2	Antioxidants	2	Gases, water vapor, other molecules	2
Polypropylene	2	Antioxidants, lubricants	4	Gases, water vapor	1
Rubber polymers					
Natural and related synthetic	5	Heavy metal salts, lubricants, reducing agents	3	Gases, water vapor	3
Butyl	3	Heavy metal salts, lubricants, reducing agents	1	Gases, water vapor	2
Silicone	2	Minimal	5	Gases, water vapor	1

[a] Approximate scale of 1 to 5 with "1" as the lowest.

Plastic

Thermoplastic polymers have been established as packaging materials for sterile preparations such as large-volume parenterals, ophthalmic solutions and, increasingly, for small-volume parenterals. For such use to be acceptable a thorough understanding of the characteristics, potential problems and advantages for use must be developed. A historical review of these factors relative to pharmaceuticals has been prepared by Autian.[11] A recent discussion of polymers for IV solutions has been published by Lambert.[12] Autian stated that three principal problem areas exist in using these materials; namely,

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.
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 selected 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 the loss of water during storage. Reverse permeation also may occur by which oxygen or other molecules may penetrate to the inside of the container and cause oxidative or other degradation of susceptible constituents. 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 being used for parenteral packaging. *Sorption* is a problem on a selective basis, that is, sorption of a few drug molecules occurs on select polymers. For example, sorption of insulin, 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. A brief summary of some of these compatibility relationships is given in Table 1.

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 flexibility of the low-density polyethylene polymer, for ophthalmic preparations, makes it possible to squeeze the side wall of the container and discharge one or more drops without introducing contamination into the remainder of the product. The flexible bags of polyvinyl chloride or select polyolefins, currently in use for large-volume intravenous fluids, have the added advantage that no air interchange is required; the flexible wall simply collapses as the solution flows out of the bag.

Most plastic materials have the disadvantage that they are not as clear as glass and, therefore, inspection of the contents is impeded. In addition, many of these materials will 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 parenterals 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. For further details see Chapter 85.

Glass

Glass is employed as the container material of choice for most SVPs. 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 thus dissolved may hydrolyze to raise the pH of the solution, catalyze reactions 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.[13]

Types—The USP has aided in this selection by providing a classification of glass; namely,

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

Type I glass is composed principally of silicon dioxide and boric oxide, with low levels of the non-network-forming oxides. It is a chemically resistant glass (low leachability) also having a low thermal coefficient of expansion.

Types II and III glass compounds are composed of relatively high proportions of sodium oxide and calcium oxide. 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 than Type I. While there is no one standard formulation for glass among manufacturers of these USP type categories, Type II glass usually 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 to dealkalize the interior surface of the container. While it remains intact, this surface will increase substantially the chemical resistance of the glass. However, repeated exposures to sterilization and alkaline detergents will 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. The latter is used only for Type II glass and is performed on the whole container, because of the dealkalized surface; the former is performed on powdered glass, which exposes internal surfaces of the glass compound. The results are based upon the amount of alkali titrated by 0.02 *N* sulfuric acid after an autoclaving cycle with the glass sample in contact with a high-purity distilled water. Thus, the *Powdered Glass Test* challenges the leaching potential of the interior structure of the glass while the *Water Attack Test* challenges only the intact surface of the container.

Selecting the appropriate glass composition is a critical facet of determining the overall specifications for each parenteral formulation.

In general, Type I glass will be suitable for all products, although sulfur dioxide treatment sometimes is used for a further increase in resistance. 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 which is buffered, has a pH below 7 or is not reactive with the glass. Type III glass usually will be suitable principally 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 a specific manufacturer.

Physical Characteristics—Some of the physical shapes of glass ampuls and vials are illustrated in Fig 3. Commercially available containers vary in size from 0.5 to 1000 mL. Sizes up to 100 mL may be obtained as ampuls and vials, and larger sizes as bottles. The latter are used mostly for intravenous and irrigating solutions. Smaller sizes are also available as cartridges. Ampuls and cartridges are drawn from glass tubing. The smaller vials may be made by mold-

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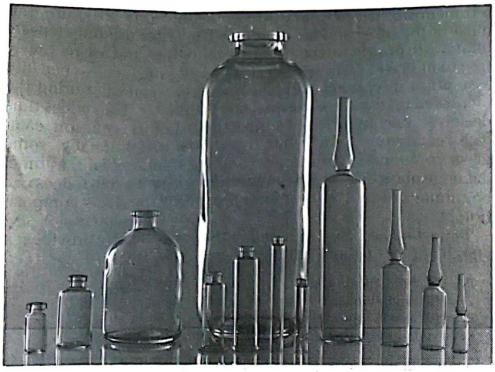


Fig 4. Various types of ampuls and multiple-dose vials for parenterals (courtesy, Kimble).

ing or from tubing. Larger vials and bottles are made only by molding. Containers made by drawing tubing are generally optically clearer and have a thinner wall than molded containers (see Fig 4). Molded containers are uniform in external dimensions, stronger and heavier.

Easy-opening ampuls 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 having a different coefficient of thermal expansion. An example of a modification of container design to meet a particular need is the double-chambered vial, under the name Univial (*Univial*), designed to contain a freeze-dried product in the lower and solvent in the upper chamber. Other examples are wide-mouth ampuls 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 having a low coefficient of thermal expansion is necessary. The container also must be transparent to permit inspection of the contents.

Preparations which 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 sometimes are 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 with aseptic care and the contents used at one time. These may range in size from 1000-mL bottles to 1-mL or less ampuls, vials or syringes. The integrity of the container is destroyed when opened so that the container cannot be closed again.

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

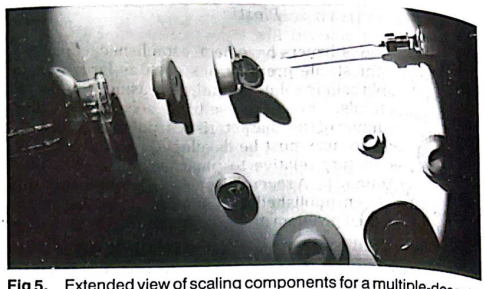


Fig 5. Extended view of scaling components for a multiple-dose vial (courtesy, West).

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 microorganisms and viruses into the contents of the vial. Because of this risk, the USP requires that all multiple-dose vials must 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 advantagious flexibility of dosage provided by multiple-dose vials, single-dose, disposable container units provide the clear advantage of greater sterility assurance and patient safety.

Rubber Closures

In order to permit introduction of a needle from a hypodermic syringe into a multiple-dose vial and provide for resealing as soon as the needle is withdrawn, each vial is sealed with a rubber closure held in place by an aluminum cap. Figure 5 illustrates how this is done. This principle also is 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 that are plasticized and mixed together at an elevated temperature on milling machines. Subsequently, 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,

Table 2—Examples of Ingredients in Rubber Closures

Ingredient	Examples
Elastomer	Natural rubber (latex)
	Butyl rubber
	Neoprene
Vulcanizing (curing) agent	Sulfur
	Peroxides
Accelerator	Zinc dibutyldithiocarbamate
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

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along with the degree of curing, affect the properties of the finished closure. Examples of rubber-closure ingredients are given in Table 2.

The physical properties to be 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 by not excessive resistance to the insertion of a needle through the closure, while minimal fragmentation of pieces of rubber should occur as the hollow shaft of the needle is pushed through the closure. While 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, examples of which are given in Table 2, pose potential compatibility interactions with product ingredients if leached into the product solution, and these effects must be evaluated.[14,15] Further, some ingredients must be evaluated for potential toxicity. In order to reduce the problem of leachables, attempts have been made to coat the product contact surfaces of closures with various polymers, the most successful being Teflon, or to treat the closures in ways considered trade secrets.

The physical shape of some typical closures may be seen in Fig 5. Most of them have a lip and a protruding flange that extends into the neck of the vial or bottle. Many disk closures are being used now, particularly in the high-speed packaging of antibiotics. Slotted closures are used on freeze-dried products to make it possible to insert the closure part way into the neck of the vial during the drying phase of the cycle. Partial insertion provides limited protection from contamination while permitting water vapor to escape from the drying product. The plunger type is used to seal one end of a cartridge. At the time of use, the plunger expels the product by a needle inserted through the closure at the distal end of the cartridge. Intravenous solution closures often have permanent holes for adapters of administration sets; irrigating solution closures usually are designed for pouring.

Production Facilities

A product having components of the best quality quickly may become totally unacceptable if the environment in which it is processed is contaminated or if the manufacturing procedure is not carried out properly. Therefore, the production facilities and the procedure used in processing the product must meet standards adequate for the task. The nearer these standards approach perfection, the better and safer should be the product.

Arrangement of Area

The production area can be considered in terms of five functional areas: the cleanup area, the compounding area, the aseptic area, the quarantine area and the finishing or packaging area. All of these should be designed and constructed for cleaning ease, appropriate environmental control, efficient operation and personnel comfort. The extra requirements for the aseptic area are designed to provide an environment where, for example, an injection may be exposed to the environment for a brief period during subdivision from a bulk container to the individual-dose containers without becoming contaminated. Contaminants such as dust, lint and microorganisms normally are found floating in the air, lying on counters and other surfaces, on clothing and body surfaces of personnel, in the exhaled breath of personnel and deposited on the floor. The design and control of an aseptic area is directed toward so reducing the presence of these contaminants that they are no longer a hazard to aseptic filling. Although the aseptic area must be adjacent to support areas so that 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, airlock pass-throughs, ports of various types or plastic curtains. Figure 6 shows an example of a floor plan 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 7 shows an adjacent arrangement with the utilization of a through-the-wall port for passage of a filtrate into the critical aseptic filling room.

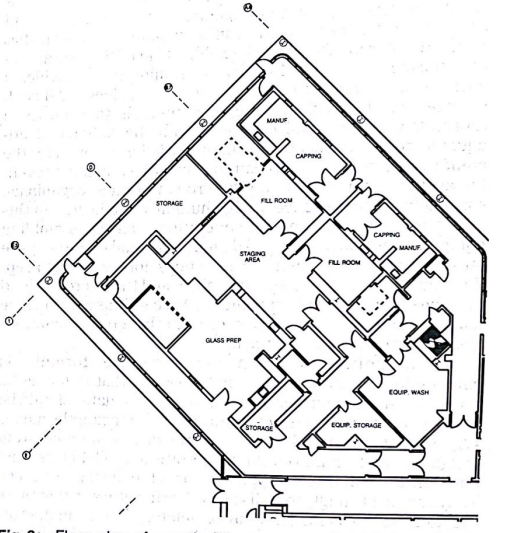


Fig 6. Floor plan of aseptic filling rooms and staging room with adjacent support areas (courtesy, Glaxo).

Flow Plan—In general, the components for a parenteral product flow from the stockroom for released components, either to the compounding area, as for ingredients of the formula, or to the cleanup area, as for containers and equipment. See Fig 8 for a process-flow diagram. 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 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 normally is interrupted after leaving the aseptic area for subjection to the sterilization process. After the results from all tests are known and the product has been found to comply with its release specifications, it passes to the finishing area for final labeling and packaging. 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 convey the components

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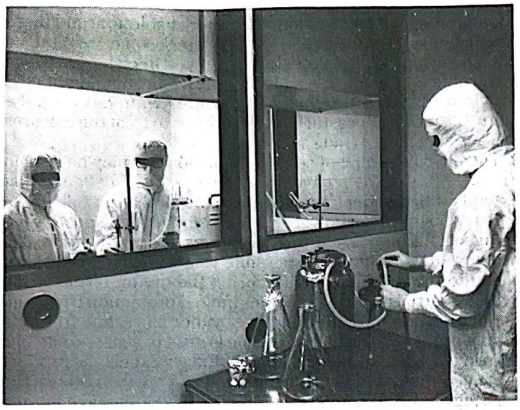


Fig 7. Product filtration from the aseptic staging room through a port into the aseptic filling room (courtesy, The University of Tennessee College of Pharmacy).

from one area to another with little or no handling by operators.

Cleanup Area—The cleanup area is constructed to withstand moisture, steam and detergents. The ceiling, walls and floor should be constructed of impervious materials so that moisture will run off and not be held. One of the "spray-on-tile" finishes with a vinyl or expoxy sealing coat 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 that the heat and humidity will be removed for the comfort of personnel. Precautions must be taken to prevent the accumulation of dirt and the growth of microorganisms, especially in the presence of high humidity and heat. In this area preparation for the filling operation, such as assembling equipment, is undertaken. Adequate sink and counter space must be provided. While this area does not need to be aseptic, it must be cleanable and kept clean and the microbial load must be monitored and controlled. Precautions also must be taken to prevent deposit of particles or other contaminants on clean containers and equipment.

Compounding Area—In this area the formula is compounded. Although it is not essential that this area be aseptic, control of microorganisms and particulates should be more stringent than in the cleanup area. For example, means may need to be 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 that there are no catch areas for dirt to accumulate. The ceiling, walls and floor should be constructed similar to those for the cleanup area.

Aseptic Area

This area requires construction features designed for maximum microbial and particulate control. The ceiling, walls and floor must be sealed so that 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 that 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 and the product fed into the area through hose lines. Figure 7 shows such an arrangement. Proper sanitization is required if the tanks must be moved in. Mechanical equipment that is located in the aseptic area should be housed as completely as possible within a stainless-steel cabinet in order to seal the operating parts and their dirt-producing tendencies from the aseptic environment. Mechanical parts that will contact the parenteral product should be demountable so that they can be sterilized.

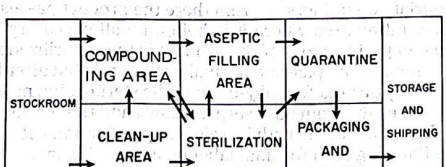


Fig 8. Process-flow diagram.

Personnel entering the aseptic area should enter only through an airlock. They should be attired in sterile coveralls with sterile hats, masks, goggles and foot covers. 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 somewhat 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.

Air Cleaning

The air in these areas can be one of the greatest sources of contamination. It need not be, however, because several methods are available for providing clean air that is essentially free from dirt particles and microorganisms.

To provide such air, it must be cleaned thoroughly of all contaminants. This may be done by a series of treatments that will vary somewhat from one installation to another. One such series is air from the outside first is passed through a prefilter, usually of glass wool, cloth or shredded plastic, to remove large particles. Then it is treated by passage through an electrostatic precipitator (Suppliers: *Am Air, Electro-Air, Sturtevant*). 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 (high efficiency particulate air) filter having an efficiency of at least 99.97% in removing particles of 0.3 μm and larger, based on the DOP (Dioctyl phthalate) test (Suppliers: *Am Air, Cambridge, Flanders*).

For personnel comfort, air conditioning and humidity control should be incorporated into the system. Another system, the Kathabar system (*Surface Combustion*), cleans the air of dirt and microorganisms by washing it in an antiseptic solution and, at the same time, controls the humidity. The clean, aseptic air is introduced into the aseptic 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 Environments—The required environmental control of aseptic areas has been made possible by the use of laminar airflow enclosures. Laminar airflow provides a total sweep of a confined space because the entire body of air moves with a uniform velocity, usually 90 ft/min, ± 20% along parallel lines, 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 arrangement for the direction of airflow can be horizontal (see Fig 9) or vertical (see Fig 10), and may involve a limited area such as a workbench or an entire room.

Figure 10 shows a vial-filling line protected with vertical laminar airflow from ceiling-hung HEPA filters. Plastic curtains are installed to maintain the laminarity of airflow to below the filling line and to circumscribe the critical filling portion of the line. The area outside the curtains can be

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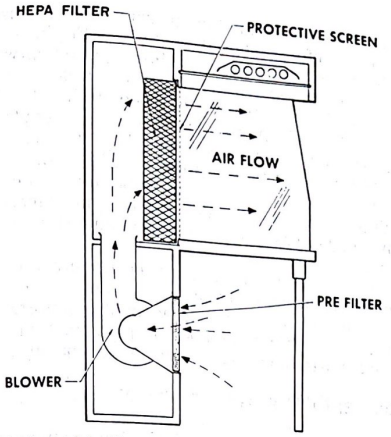


Fig 9. Horizontal laminar-flow workbench (courtesy, adaptation, Sandia).

maintained at a slightly lower level of cleanliness than that inside.

Laminar flow of HEPA-filtered air is capable of meeting the standard for a Class 100 clean room as defined by Federal Standard 209E,[16] which states that such an environment contains no more than 100 particles/ft^3 of 0.5 μm and larger in size. Thus, in Fig 10, the area within the curtains should be a Class 100 environment but the area outside may be Class 10,000 or cleaner, defined on the same basis. Today, it is accepted that critical areas of processing, wherein the product or product contact surfaces may be exposed to the environment, even for a brief period of time, should meet Class 100 clean room standards.

It must be borne in mind that any contamination introduced upstream by equipment, arms of the operator or leaks in the filter will be blown downstream. In the instance of horizontal flow this may be to 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 microorganisms, 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 operations involving such contaminants 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. Vertical flow has been recommended for sterility-testing procedures.

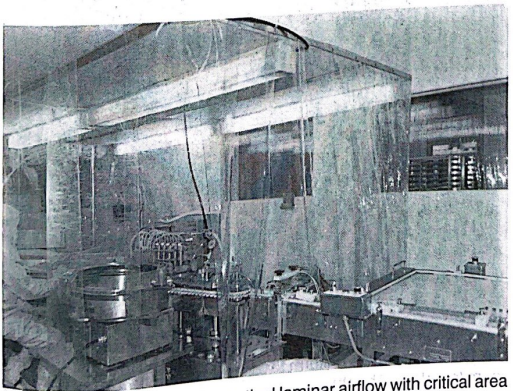


Fig 10. Vial filling line under vertical laminar airflow with critical area enclosed within plastic curtains (courtesy, Merck).

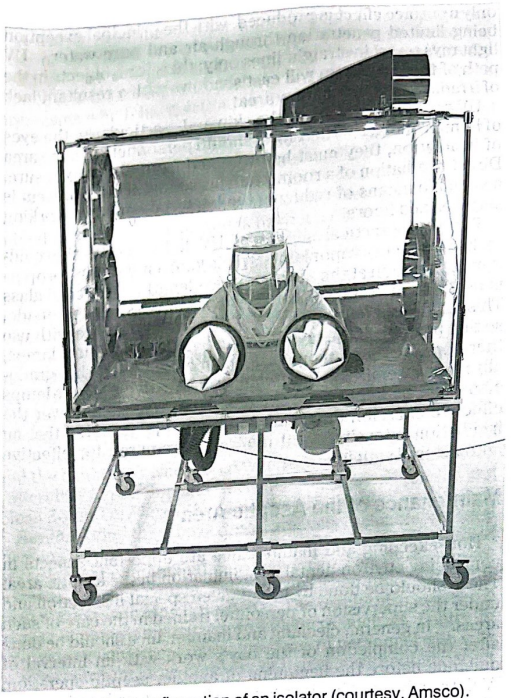


Fig 11. One configuration of an isolator (courtesy, Amsco).

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 operators.

Therefore, laminar-flow work areas should be protected by being located within controlled environments. Personnel should be attired for aseptic processing as described below. All movements and processes should be planned carefully to avoid the introduction of contamination upstream of the critical work area. Checks of the airstream should be performed initially and at regular intervals to be sure no leaks have developed through or around the HEPA filters.

Workbenches and other types of laminar-flow enclosures are available from several commercial sources (Suppliers: *Air Control, Atmos-Tech, Baker, Clean Air, Clestra, EACI/Envirco, Flanders, Laminaire, Liberty, Veco*).

Isolation (barrier) technology is a relatively new approach designed to isolate aseptic operations from personnel. Figure 11 shows the structure of one configuration of an isolator. As can be seen, the operations are performed within transparent, plastic, sealed walls with the operator, in this instance, working through gloves and a half-suit. These isolation work stations are presterilized, usually with peracetic acid or hydrogen peroxide vapor, and sterile supplies are introduced from sterilizing modules through uniquely engineered transfer ports. Results from the use of these units in controlling contamination during sterility testing have reduced false positives to essentially zero. Studies are being undertaken to evaluate these units for aseptic production operations.[17]

Ultraviolet Radiation

Ultraviolet (UV) light rays have an antibacterial action, thereby producing a disinfectant action on directly irradiated surfaces. Since these rays cannot penetrate most materials,

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only a surface effect is produced, with the principal exception being limited penetration through air and pure water. UV light rays travel in straight lines only; therefore, objects in the path of the light beam will cast shadows with a resultant lack of irradiation in the shadow area.

UV rays are irritating to the skin and, particularly, the eyes of human beings. Therefore, should personnel be in the area of irradiation, they must be protected from direct exposure. Direct irradiation of a room when personnel are not present is a valuable means of reducing the bacterial count on working surfaces and floors.

The best practical source of UV light rays is the cold-cathode mercury vapor lamp. This lamp emits a high proportion of radiation at the 253.7 nm wavelength. A special glass is used for the tube so that the rays will pass to the outside. This glass gradually will change in crystal structure with use so that passage of the rays is gradually reduced. Such lamps, therefore, rarely burn out as do visible-light lamps but gradually reach an emission level which is ineffective. These lamps also must be kept clean, for dust and grease will lower the effective emission drastically. It generally is stated that an irradiation intensity of 20 $\mu w/cm^2$ is required for effective antibacterial activity.

Maintenance of the Aseptic Area

Housekeeping and maintenance are important aspects in controlling environmental contamination in the aseptic area. These should be done by crews given special instruction and under the supervision of personnel trained in the care of such areas. In general, cleaning and maintenance should be done after the completion of the day's work with an interval of quietude before the beginning of another aseptic operation. With the advent of laminar flow of HEPA-filtered air the rigors of cleaning have been reduced since the clean airflow continuously "sweeps" the area clean. All maintenance equipment should be selected for its effectiveness and freedom from lint-producing tendencies and should be reserved for use in aseptic areas only.

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 they show symptoms of a head cold, allergies or similar illness, they should not be permitted in the aseptic area until their recovery is complete. However, a healthy person with the best personal hygiene still will shed large numbers of viable and nonviable particles from body surfaces. This natural phenomenon creates continuing problems when personnel are present in clean rooms, but 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 be sure training has been effective, before they 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 who 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 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. The uniform usually consists of coveralls for both men and women, hoods to completely cover the hair, face masks and Dacron or plastic boots (Fig 12). Sterile rubber gloves also are required for aseptic operations, preceded by thorough scrubbing of the hands with a disinfectant soap. In addition, goggles may be required to complete the coverage of all skin areas.

Dacron or Tyvek uniforms are used usually, 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.

Environmental Control Evaluation

As evidenced by the above discussion, manufacturers of sterile products use extensive means to control the environment so that these critical 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 nonviable 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 they exceed selected alert or action levels, a plan of action must be put into operation to determine if or what corrective measures are required.

The tests used generally measure either the particles in a volume of sampled air or the particles that are settling or are present on surfaces. A volume of air measured by an elec-



Fig 12. Appropriate uniform for operators entering an aseptic filling room (courtesy, Abbott).

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tronic particle counter will detect all particles instantly, but not differentiate between viable and nonviable ones. However, because of the need to control the level of microorganisms in the environment in which sterile products are processed, it also is necessary to detect viable particles. These usually are less in number than nonviable ones and are only detectable as colony forming units (CFUs) after a suitable incubation period at, for example, 30 to 35°C for up to 48 hours.

Locations for sampling should be planned to reveal potential contamination levels which 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. 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 size of 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 to be increased; for example, in Class 100 areas, Whyte and Niven,[18] suggest that the sample should be at least 30 ft^3 and, probably, much more. They also suggest 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.

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 (Suppliers: *Climet, Met One, Particle Measuring, Royco*). These instruments not only count particles but also provide a size distribution based on the magnitude of the light scattered from the particle.

Several air-sampling devices are used to obtain a count of microorganisms in a measured volume of air. A slit-to-agar (STA) sampler (Suppliers: *Mattson-Garvin, New Brunswick*) draws by vacuum a measured volume of air through a narrow opening causing the air to impact on the surface of a slowly rotating nutrient agar plate. Microorganisms adhere to the surface of the agar and grow into visible colonies which are counted as CFUs, since it is not known whether the colonies arise from a single microorganism or a cluster. A centrifugal sampler (Supplier: *Biotest*) pulls air into the sampler by means of a rotating propeller and slings the air by centrifugal action against a peripheral nutrient agar strip. The advantages of this unit are that it can be disinfected easily and is portable so that it can be hand-carried wherever needed. These two methods are used quite widely.

A widely used method for microbiological sampling consists of the exposure of nutrient agar culture plates to the settling of microorganisms from the air. This method is very simple and inexpensive to perform but will detect only those organisms which have settled on the plate; therefore, it does not measure the number of microorganisms in a measured volume of air. 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.

The number of microorganisms on surfaces can be determined with nutrient agar plates having a convex surface (*Rodac Plates*). With these it is possible to roll the raised agar surface over flat or irregular surfaces to be tested. Organisms will be picked up on the agar and will grow during subsequent incubation. This method also can be used to assess the number of microorganisms 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 microorganisms during processing. Whenever 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 to be accepted will be found in Section ⟨1116⟩ "Microbial Evaluation and Classification of Clean Rooms and Clean Zones" in *Pharm Forum 18:*4048, 1992.

Results from the above tests are very 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 also may 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.

Media Fill—An evaluation which is not strictly an environmental test, but which includes an evaluation of the environment along with the process, the operators and the equipment, is the "media fill." Sterile trypticase soy broth is filled into sterile containers under conditions simulating as closely as possible those characteristics of a filling process for a product. The entire lot, normally at least 3000 units, is then incubated at a suitable temperature, usually 20 to 25°, for at least 14 days and examined for the appearance of growth of microorganisms. If growth occurs, contamination has entered the container(s) during the processing. To pass the test not more than 0.1% of the units may show growth. This evaluation also has been used as a measure of the proficiency of an individual operator. This test is a very stringent evaluation of the efficiency of an aseptic filling process and, by many, is considered to be the most evaluative test available.

Production Procedures

The processes required for preparing sterile products constitute a series of events initiated with the securing of approved raw materials (drugs, excipients, vehicles, etc) and primary packaging components (containers, closures, etc) and ends with the sterile product sealed in its dispensing package. Each step in the process must be controlled very carefully in order that the product will have its required quality. To assure the latter, each process should be validated to be sure that it is accomplishing what it is intended to do. For example, an autoclave sterilization process must be validated by providing data showing that it effectively kills resistant forms of microorganisms; or, a cleaning process for rubber closures should provide evidence that it is cleaning closures to the required level of cleanliness. The validation of processes requires an extensive and intensive effort to be successful, and is an integral part of CGMP requirements.

In the following sections the production procedures used in preparing sterile drug products will be discussed.

Cleaning Containers and Equipment

Containers and equipment coming in contact with parenteral preparations must be cleaned meticulously. It is obvious that if this were not so, all other precautions to prevent contamination of the product would be useless. It also should be obvious that even new, unused containers and equipment will be 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. Much greater contamination must be removed from previously used equipment before it will be suitable for reuse. Equipment should be reserved exclusively for use only with

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parenteral preparations and, where conditions dictate, only for one product in order to reduce the risk of contamination.

A variety of machines are available for cleaning containers for parenteral products. These vary in complexity from a small rotary rinser (Fig 13) to large automatic washers capable of processing several thousand containers an hour (Fig 14). The selection of the particular type will be determined largely by the physical type of containers, their condition with respect to contamination and the number to be processed in a given period of time.

Characteristics of Machinery—Regardless of the type of cleaning machine selected, certain fundamental characteristics usually are 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 the flow should be such that it can leave the container opening without accumulating and producing 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 for a planned sequence alternating very hot and cool treatments. The final treatment should be an effective rinse with water of a quality equivalent to WFI.
4. All metal parts coming in contact with the containers and with the treatments should be constructed of stainless steel or some other noncorroding and noncontaminating material.

Treatment Cycle—The cycle of treatments to be employed will vary with the condition of the containers to be cleaned. In general, loose dirt can be removed by vigorous rinsing with water. Detergents rarely are used for new containers because of the risk of leaving detergent residues. However, a thermal-shock sequence in the cycle usually is 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, particularly if used for a dry powder. In all instances the final rinse, whether air or WFI, must be ultraclean so that 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, as can be seen stacked on the right of Fig 14.

Machinery for Containers—The machinery available for cleaning containers embodies the above principles but varies in the mechanics by which it is accomplished. In one approach, 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. Such a small-scale machine is pictured in Fig 14. A washer capable of cleaning hundreds of containers an hour, shown in Figure 14, uses a row of jet tubes across a conveyor belt. The belt moves the inverted containers past the programmed series of treatments and discharges the clean containers into a sterilizing oven (not shown), which ultimately discharges them through a wall into a clean room for filling. Another type of machine is the rack-loading washer. Stainless-steel racks are designed to fit over the open ends of ampuls or vials as configured in trays of shipping cartons or blister packs. Inverting the trays permits the containers to slide into the racks so that they can be handled by the quantity in the tray, as shown in Fig 15. The clean containers may be transferred directly to the conveyor of a sterilizating tunnel (as shown), or they may be placed in stainless-steel boxes for subsequent dry-heat sterilization and storage. A continuous automated line operation is shown in Figure 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 through the wall into the filling clean room.

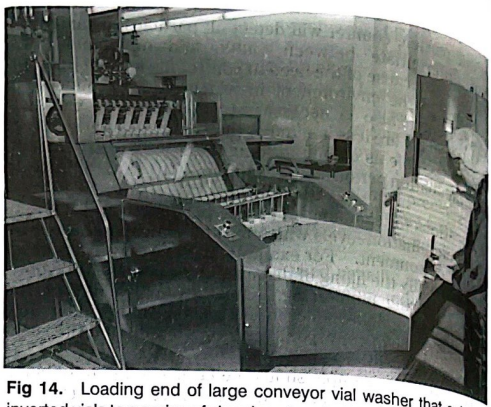


Fig 14. Loading end of large conveyor vial washer that subjects inverted vials to a series of cleaning steps before delivery from the far end of the washer. Note the vials in plastic blister packs at right of operator (courtesy, Merck).

Handling after Cleaning—The wet, clean containers must

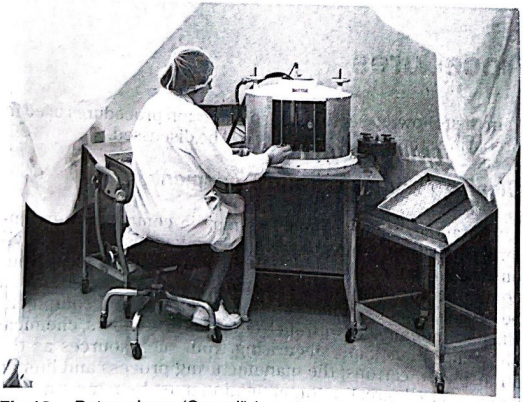


Fig 13. Rotary rinser (Cozzoli) in a clean environment provided by vertical laminar airflow within a curtained enclosure (courtesy, Ciba-Geigy).



Fig 15. Cleaning vials with rack-loading washer, permitting handling vials by a full rack. After multiple-washing treatments, the racks are placed directly on the conveyor belt of the hot-air sterilizing tunnel (courtesy, Merck).

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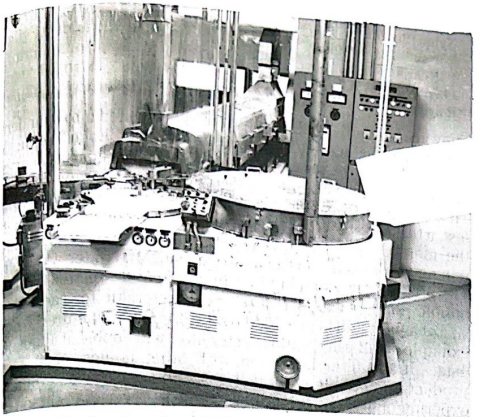


Fig 16. Continuous automatic line operation for vials from a rotary rinser through a sterilizing tunnel with vertical laminar-airflow protection of clean vials (courtesy, Abbott).

be handled in such a way that contamination will not be reintroduced. A wet surface will collect contaminants much more readily than will a dry surface. For this reason wet, rinsed containers must be protected, such as by a laminar flow of clean air until covered, as within a stainless-steel box or within a sterilizing tunnel. Although not clearly visible in each instance, the wet, clean containers in Figs 13–16 were so protected. 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 generally has been considered to be adequate also to destroy pyrogens, but 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. In Fig 16, the clean, wet containers are protected by filtered laminar-flow air from the rinser through the tunnel and until they are delivered to the filling line.

Closures—Rubber closures are coated with lubricant from the molding operation. In addition, the rough surface and electrostatic attraction tend to hold debris. Also, the surface "bloom" from migrated inorganic constituents of the compound must be removed. The recommended procedure calls for gentle agitation in a hot solution of a mild water softener. The closures are removed from the solution and rinsed several times, or continuously for a prolonged period, with filtered WFI. The rinsing is to be done in a manner which will flush away loosened debris. The wet closures are carefully protected from pick up of environmental contamination, are sterilized, usually by autoclaving, and stored in closed containers until ready for use. This cleaning and sterilizing process must 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 them. 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°.

The equipment used for washing large numbers of closures is usually an agitator or horizontal basket-type automatic washing machine. Because of particulate generation from the abrading action of these machines, some heat the closures in kettles in detergent solution and follow with prolonged flush rinsing. The final rinse always should be with low particulate WFI.

Equipment—The details of certain prescribed techniques for cleaning and preparing equipment, as well as of containers and closures, have been presented elsewhere.[19] Here, a few points will be emphasized.

All equipment should be disassembled as much as possible to provide access to internal structures. For thorough cleaning, surfaces should be scrubbed thoroughly with a stiff brush using an effective detergent, paying particular attention to joints, crevices, screw threads and other structures where debris is apt to collect. Exposure to a stream of clean steam will aid in dislodging residues from the walls of stationary tanks, spigots, pipes and similar structures. Thorough rinsing with distilled water should follow the cleaning steps. Large stationary tanks, such as those shown in Fig 17, should be protected as much as possible from contamination after cleaning but should be rinsed thoroughly again with distilled water or WFI prior to reuse.

Because of the inherent variation in the manual cleaning of tanks and other large equipment items, together with the need to validate the process, an automated, usually computer-controlled, system has been developed called "cleaning in place" (CIP).[20] Such an approach involves designing the system, normally of stainless steel, with smooth, rounded internal surfaces and without crevices. That is, for example, with welded rather than threaded connections. The cleaning is accomplished with the scrubbing action of high-pressure spray balls or nozzles delivering hot detergent solution from tanks captive to the system. Thorough rinsing with WFI follows and is accomplished within the same system. The system often is extended to permit sterilizing in place (SIP) as well.[21]

Rubber tubing, rubber gaskets and other rubber parts may be washed in a manner such as described for rubber closures. Thorough rinsing of tubing must be done by passing WFI through it. 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 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 do not vary from those used routinely by qualified pharmacists. However, selected aspects will be mentioned for emphasis.

A master formula would have been developed and on file. Each batch formula sheet should be prepared from the master and confirmed for accuracy. All measurements of quantities

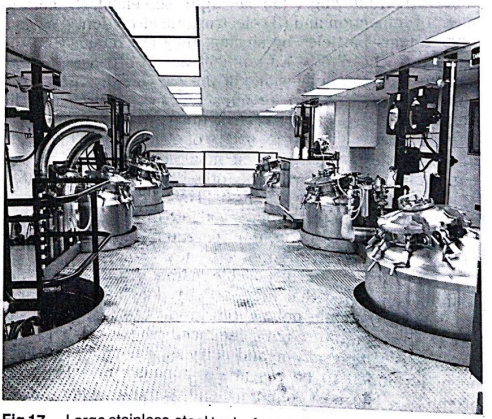


Fig 17. Large stainless-steel tanks for product preparation showing mezzanine access level (courtesy, Abbott).

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should be made as accurately as possible and checked by a second qualified person. Frequently, today, the formula documents are generated by a computer and the measurements of quantities of ingredients computer controlled. Although most liquid preparations are dispensed by volume, they are prepared by weight, since weighings 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 significantly dilute the product 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 that 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. Parenteral emulsions have been reviewed by Singh and Ravin.[22] 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.

The formulation of a stable product is of paramount importance. Certain aspects of this have been mentioned in the discussion of components of the product. Exhaustive coverage of the topic is not possible within the limits of this text, but further coverage is provided in Chapter 83. It should be mentioned here, however, 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 a solution. A high degree of clarification is termed "polishing" a solution. This term is used when particulate matter down to approximately 2 μm in size is removed. A further step, removing particulate matter down to 0.2 μm in size, would eliminate microorganisms and would accomplish "cold" sterilization. A solution having 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 the following: (1) sieving or screening, (2) entrapment or impaction and (3) electrostatic attraction. 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 for parenteral solutions because of their particle-retention effectiveness, nonshedding property, nonreactivity and disposable characteristics. However, it should be noted that nonreactivity does not apply in all cases. For example, polypeptide products may show considerable adsorption through some membrane filters, but those composed of polysulfone and polyvinylidine difluoride have been developed to be essentially nonadsorptive for these products. The most common membranes are composed of

Cellulose ester (Suppliers: *Gelman, Millipore, Sartorius, Schleicher Seitz*).
Nylon (Supplier: *Pall*).
Polysulfone (Supplier: *Gelman*).
Polycarbonate (Supplier: *Nuclepore*).
Polyvinylidene difluoride (Supplier: *Millipore*).
Polytetrafluoroethylene (Teflon) (Supplier: *Millipore*).

They are available as flat membranes or pleated into cylinders 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 microorganisms. This integrity test usually is performed as the *bubble-point test*, a test 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 pressure at which bubbles first appear downstream is the bubble point. This pressure is characteristic for each pore size of a filter and is provided by the filter manufacturer. For example, a 0.2 μm cellulose ester filter will bubble at about 50 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 pressure, the filter is defective, probably due to a puncture or tear, and should not be used. As the surface area of filters becomes large, diffusion of air through the water-filled pores tends to obscure the bubble point. Therefore, a diffusion, or pressure hold, test has been developed as an integrity test for filters with large surface areas. Particulars are obtainable from the filter manufacturer.

While membrane filters are disposable, and thus discarded after use, the holders must be cleaned thoroughly between uses. Today, clean, sterile, pretested, disposable assemblies for small as well as large volumes of solutions are available commercially. Other characteristics of these filters, important for a full understanding of their use, are given in Chapter 84 and in a review article.[23]

Filling

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 usually is called an "aseptic fill" and is validated with media fills (see page 1537). During the filling operation, the product must be transferred from a bulk container and subdivided into dose containers. This operation exposes the sterile product to the environment, equipment and manipulative technique of the operator until it can be sealed in the dose container. Therefore, this operation is carried out with a minimum exposure time in the aseptic filling area where maximum protection is provided. Additional protection may be provided by filling under a blanket of HEPA-filtered laminar-flow air within the aseptic area.

Normally, the compounded product is in the form of either a liquid or a solid. A liquid is more readily subdivided uniformly and introduced into a container having a narrow mouth than is a solid. Mobile, nonsticking 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 which is introduced into the container. The size of the delivery tube will vary from that of about a 20-gauge hypodermic needle to a tube ½ 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 permit air to leave the container as the liquid enters. The delivery tube should be as large in diameter as possible in order to reduce the resistance to the flow of the liquid. For smaller volumes of liquids, the delivery usu-

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ally 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. A sliding piston valve would be used for heavy, viscous liquids. Other mechanisms include the turn of an auger in the neck of a funnel or the oscillation of a rubber diaphragm. For large volumes the quantity delivered usually 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 ampul 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 ampul 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 nonreactive materials such as borosilicate glass or stainless steel. In addition, they should easily be demountable for cleaning and sterilization.

Because of the concern for particulate matter in injectable preparations, a final filter often is inserted in the system between the filler and the delivery tube, as shown in Fig 18. Most frequently this is a membrane filter, having a porosity of approximately 1 μm and treated to have a hydrophobic edge. This is necessary to reduce the risk of rupture of the membrane due to filling pulsations. It should be noted that the insertion of the filter at this point should collect all particulate matter generated during the process. Only that which may be found in inadequately cleaned containers or picked up from exposure to the environment after passage through the final filter potentially remain as contaminants. However, the filter does cushion liquid flow and reduces the efficiency of drop retraction from the end of the delivery tube, sometimes making it difficult to control delivery volume as precisely as would be possible without the filter.

Liquids—The filling of a small number of containers may be accomplished with a hypodermic syringe and needle, the liquid being drawn into the syringe and forced through the needle into the container. A device for providing greater speed of filling is the Cornwall Pipet (*Becton Dickinson*). This has a two-way valve between the syringe and the needle and a means for setting the stroke of the syringe so that the same volume will be delivered each time. Clean, sterile, disposable assemblies (Suppliers: *Burron, Pharmaseal*) operating on the same principle have particular usefulness in hospital pharmacy operations.

Mechanically operated instruments substitute a motor for the operator's hand in the previous devices described. Thereby, a much faster filling rate can be achieved. By careful engineering, the stroke of the syringe can be repeated precisely, and so, once a particular setting has been calibrated to the delivery, high delivery precision is possible. However, the speed of delivery, the expansion of the rubber tubing connecting the valve with the delivery tube and the rapidity of action of the valves can affect the precision of delivery. A filling machine employing a piston valve is shown in Fig 18. Stainless-steel syringes are required with viscous liquids because glass syringes are not strong enough to withstand the high pressures developed during delivery.

When high-speed filling rates are desired but accuracy and precision must be maintained, multiple filling units often are joined together in an electronically coordinated machine, such as shown in Fig 19. 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, there is some sacrifice of filling accuracy.

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

To insure delivery of the labeled volume to the patient, the USP provides a table of suggested fill volumes to include a slight excess over labeled volume.

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 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. Because of these factors, the tolerances permitted for the content of such containers must be relatively large. Suggested tolerances can be found in the USP.

Some sterile solids are subdivided into containers by individual weighing. A scoop usually is provided to aid in approximating the quantity required, but the quantity filled into the container finally is weighed on a balance. This is a slow process. When the solid is obtainable in a granular form so that 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 which has been calibrated in terms of the weight desired. In the machine shown in Fig 20 an adjustable cavity in the rim of a wheel is

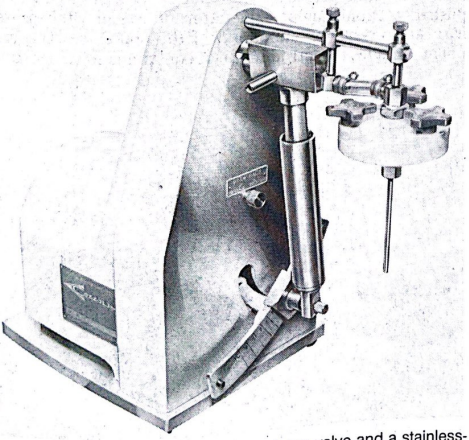


Fig 18. Filling machine employing a piston valve and a stainless-steel syringe (courtesy, Cozzoli).

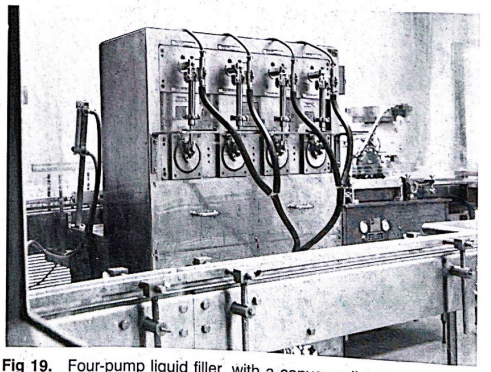


Fig 19. Four-pump liquid filler, with a conveyor line for vials protected by a vertical laminar airflow and plastic curtain; note the automatic stoppering machine on the right within the curtain (courtesy, Abbott).

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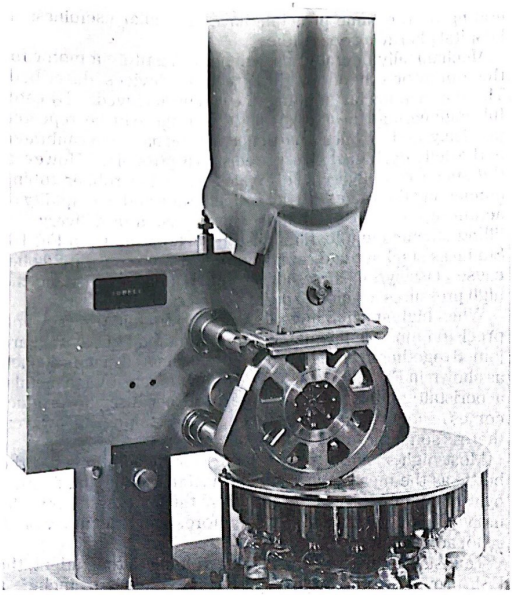


Fig 20. Accofil vacuum powder filler (courtesy, Perry).

filled by vacuum and the contents held by vacuum until the cavity is inverted over the container. The solid material then is discharged into the container by the use of sterile air. Another machine employs an auger in the stem of a funnel at the bottom of a hopper. The granular material is placed in the hopper. By controlling the size of the auger and its rotation, a regulated volume of granular material can be delivered from the funnel stem into the container. Such a machine is shown in Fig 21.

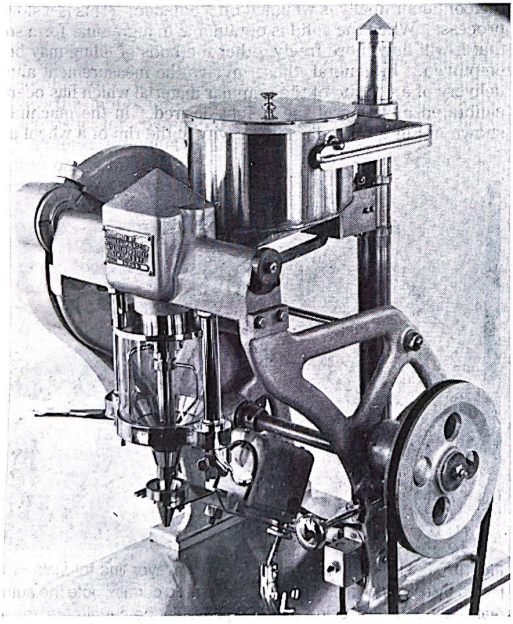


Fig 21. Auger-type powder filler (courtesy, Chase-Logeman).

Sealing

Ampuls—Filled containers should be sealed as soon as possible to prevent the contents from being contaminated by the environment. Ampuls 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 ampul 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 ampul neck must be heated evenly on all sides. This may be accomplished by means of burners on opposite sides of stationary ampuls or by rotating the ampul in a single flame. Care must be taken to properly adjust the flame temperature and the interval of heating to obtain complete closing of the opening with a bead of glass. Excessive heating will result in the expansion of the gases within the ampul against the soft bead seal and cause a bubble to form. If it bursts, the ampul 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 ampul is called a "leaker."

Pull-seals are made by heating the neck of the ampul below the tip, leaving enough of the tip for grasping with forceps or other mechanical devices. The ampul 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 ampul, which continues to rotate. The small capillary tube thus formed is twisted closed. Pull-sealing is slower, but the seals are more sure than tip-sealing. Fig 22 shows a machine combining the steps of filling and pull-sealing ampuls.

Powder ampuls or other types having a wide opening must be sealed by pull-sealing. Were these sealed by tip-sealing, the very large bead produced would induce glass strain with subsequent fracture at the juncture of the bead and neck wall. Fracture of the neck of ampuls during sealing also may occur if wetting of the necks occurred at the time of filling. Also, wet necks increase the frequency of bubble formation. If the product in the ampul is organic in nature, wet necks also will result in unsightly carbon deposits from the heat of sealing.

In order to prevent decomposition of a product, it is sometimes necessary to displace the air in the space above the product in the ampul with an inert gas. This is done by introducing a stream of the gas, such as nitrogen or carbon dioxide, during or after filling with the product. Immediately thereafter the ampul 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.

Vials and Bottles—These 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

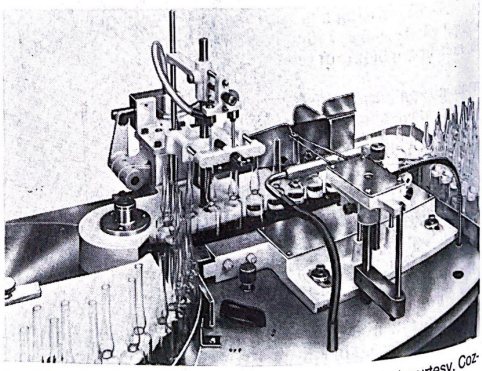


Fig 22. Automatic filling and pull-sealing of ampuls (courtesy, Cozzoli).

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than with ampuls. Therefore, a covering should be provided for such containers except for the minimal time required for filling and for the actual introduction of the rubber closure. During the latter critical time the open containers should be protected from the ingress of contamination, preferably with a blanket of HEPA-filtered laminar airflow, as shown in Figs 10 and 19.

The closure must fit the mouth of the container snugly enough so that its elasticity will permit adjustment 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. Closures preferably are inserted mechanically using an automated process, especially with high-speed processing. To reduce friction so that the closure may slide more easily through a shute and into the container opening, the closure surfaces often are halogenated or treated with silicone. When the closure is positioned at the insertion site, it is mechanically pushed into the container opening (see Fig 23). 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.

Rubber closures are held in place by means of aluminum caps. The caps cover the closure and are crimped under the lip of the vial or bottle to hold them in place (see Fig 5). 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 assure the integrity of the contents as to sterility and other aspects of quality.

The aluminum caps are so designed that 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 which 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 overlayed with a solid aluminum disk is placed between an inner and outer aluminum cap, thereby providing a seal of the hole through the closure. These are called triple-layered aluminum caps.

Single-layered aluminum caps may be applied by means of a hand crimper known as the Fermpress (Suppliers: *West, Wheaton*). Double- or triple-layered caps require greater force for crimping; therefore, heavy-duty mechanical crimpers (see Fig 24) are required (Suppliers: *Bosch, Cozzoli, Perry, West, Wheaton*).

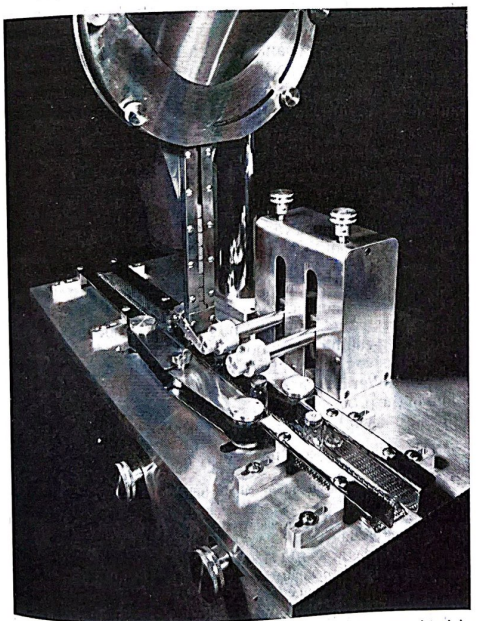


Fig 23. Mechanical device for inserting rubber closures in vials (courtesy, Perry).

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 the filling and sealing have been completed. Since this usually involves a thermal process, due consideration must be given to the effect of the elevated temperature upon the stability of the product. Many products, both pharmaceutical and biological, will be affected adversely by the elevated temperatures required for thermal sterilization. Heat-labile products must, therefore, be sterilized by a nonthermal method, usually by filtration through bacteria-retaining filters. Subsequently, all operations must be carried out in an aseptic manner so that contamination will not be 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 difficult and it has been accepted that currently, under the best controlled conditions, a sterility assurance level (SAL) of 10^{-3} is the maximum achievable. On the other hand, technical advances in aseptic processing, including improved automation, use of barrier systems, formulations to include antimicrobial effects and combinations of limited sterilization with aseptic processing, have improved the sterility-assurance levels achievable. Therefore, the successes realized should encourage continued efforts to improve the SAL achievable with aseptic processing. The importance of this is that for many drug solutions aseptic processing is the only method that can be considered for preparing a sterile product.

Nonthermal methods of sterilization, such as irradiation, have been proposed for consideration. However, since there is limited understanding of the molecular transformations that may occur in drug molecules and excipients under exposure to the high-energy levels of the process, extensive research will be required to develop the knowledge needed for an adequate evaluation.

Dry-heat sterilization may be employed for a few dry solids that are not affected adversely by the high temperatures and

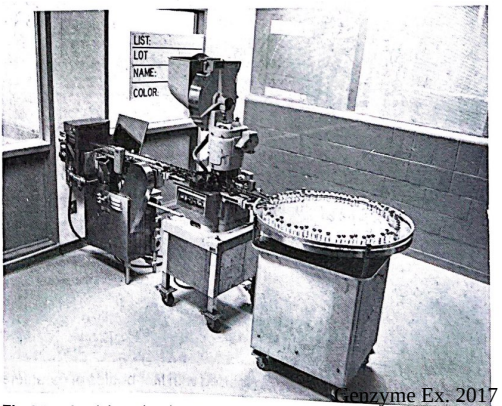


Fig 24. Applying aluminum caps to vials on a production line (courtesy, Abbott).

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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. An SAL of 10^{-6} is readily achievable with terminal autoclaving of a thermally stable product. This is a 1000-fold improvement over the currently accepted level for aseptic processing. However, it needs to be noted that for terminal sterilization, as with autoclaving, the SAL is based upon an evaluation of the lethality of the process, ie, of the probable number of viable microorganisms remaining in product units. However, for aseptic processing, the components used have been sterilized by a validated process and the SAL is based upon an evaluation of the probable number of product units that have been contaminated during the process. This difference does not alter the outcome but only the basis for evaluating the probable SAL.

Figure 25 shows liter containers of solution being loaded into an 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 controlled carefully. As mentioned previously, some injections will be affected adversely by the elevated temperature required for autoclaving. For some products, such as Dextrose Injection, the use of an autoclave designed to permit a rapid rise to sterilizing temperature and rapid cooling with water spray after the sterilizing hold-period will make it possible to use this method. It is ineffective in anhydrous conditions, such as within a sealed ampul 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 inspissation. These methods may be rendered more effective for some injections by the inclusion of a bacteriostatic agent in the product.

It should be obvious that all materials subjected to sterilization must be protected from subsequent contamination to maintain their sterile state. Therefore, they must be wrapped or covered so that microorganisms may not gain access when removed from the autoclave. Equipment and supplies are wrapped most frequently with paper and tied or sealed with special autoclave tape. The wrapping must permit penetration of steam during autoclaving but screen out microorganisms when dry. A double wrapping with lint-free parchment paper designed for such use is probably best. Synthetic fiber cloth such as nylon or Dacron also may be used for the inner wrapping. The openings of equipment subjected to dry-heat sterilization are often covered with silver–aluminum foil or with metal or glass covers. Cellulose wrapping materials are affected adversely by the high temperatures of dry-heat sterilization.

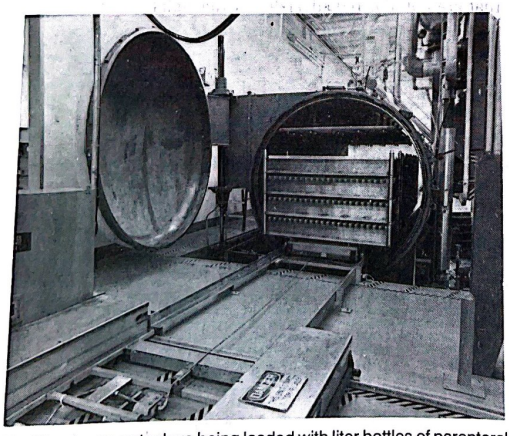


Fig 25. Large autoclave being loaded with liter bottles of parenteral solutions (courtesy, Abbott).

The effectiveness of any sterilization technique must be proved (validated) before it is employed. Since the goal of sterilization is to kill microorganisms, the ideal indicator to prove the effectiveness of the process is a resistant form of an appropriate microorganism, normally resistant spores (a biological indicator or BI). Therefore, during validation of a sterilization process, BIs of known resistance are used and numbers 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 because of the ever-present risk of an undetected, inadvertent contamination of a product or the environment.

The commercial suppliers of BIs have improved the resistance evaluation and lot-to-lot reliability of their products so that the information provided with each lot of BIs is generally reliable and useful in validation procedures.

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 will be found in Chapter 84. In addition, the USP provides suggestions concerning the sterilization of injections and related materials.

Freeze-Drying

Freeze-drying (lyophilization) is a process of drying in which water is sublimed from the product after it is frozen.[24] The particular advantages of this process are that biologicals and pharmaceuticals which are relatively unstable in aqueous solution can be processed and filled into dosage containers in the liquid state, taking advantage of the relative ease of processing a liquid. They can be dried without elevated temperatures, thereby eliminating adverse thermal effects, and stored in the dry state in which there are relatively few stability problems.

Further advantages are that these products are often more soluble and/or more rapidly soluble, dispersions are stabilized throughout their shelf life and products subject to degradation by oxidation have enhanced stability because the process is carried out in a vacuum.

However, the increased time and handling required for processing and the cost of the equipment limit the use of this process to those products which significantly have enhanced stability if stored in the dry state.

The fact that ice will sublime at pressures below 3 torr has been a long-established laboratory principle (see Chapter 19). The extensive program for freeze-drying human plasma during World War II provided the impetus for the rapid development of the process.

Freeze-drying essentially consists of

1. Freezing an aqueous product at a temperature below its eutectic temperature.
2. Evacuating the chamber, usually below 0.1 torr (100 μm Hg).
3. Subliming ice on a cold condensing surface at a temperature below that of the product, the condensing surface being within the chamber or in a connecting chamber.
4. Introducing heat to the product under controlled conditions, thereby providing energy for sublimation at a rate designed to keep the product temperature below its eutectic temperature.

Figure 26 shows such a system. The product may be fro-

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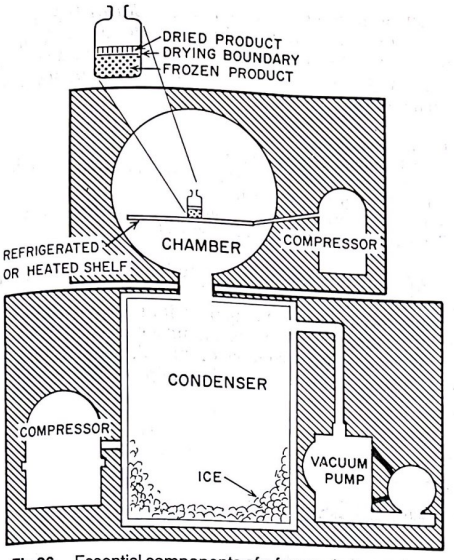


Fig 26. Essential components of a freeze-drying system.

zen on the shelf in the chamber by circulating refrigerant (usually Freon, ammonia or ethylene glycol) 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 by electric resistance coils or by circulating hot water, silicone or glycol. The process continues until the product is dry (usually 1% or less moisture), 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 and then 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.

The chambers may be equipped with hydraulic or rubber diaphragm 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 that the slots were open to the outside.

If internal stoppering is not available or containers such as ampuls are used, filtered dry air or nitrogen must be introduced to the chamber at the end of the process to establish atmospheric pressure. Then the containers must be removed and sealed under aseptic conditions. If the product is very sensitive to moisture, the environmental humidity also must be controlled until it is sealed.

Factors Affecting the Process Rate—The greater the depth of the product in the container, the longer will be the drying process. Therefore, a product to be frozen by placing the container on a refrigerated shelf (plug freezing) should be filled to a planned, limited depth. If a large volume of solution must be processed, the surface area may be increased and the depth decreased by freezing the solution on a slant or while rotating the container on an angle (shell freezing) in a liquid refrigerant bath, such as dry ice and alcohol.

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 that 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 usually 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.

This may be visualized by referring to Fig 26.

The passageways between the product surface and the condenser surface must be wide open and direct for effective operation. Therefore, the condensing surfaces in large freeze-driers are usually in the same chamber as the product. 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, their particle size and their thermal conductance will affect the rate of drying. The more solids present, the more impediment will be provided to the escape of the water vapor. The smaller the particle size, particularly the crystal size of the ice, the faster the drying generally will be. The poorer the thermal conducting properties of the solids in the product, the slower will be the rate of heat transfer through the frozen material to the drying boundary.

The rate of drying is essentially slow, most often requiring 24 hours or longer for completion. The actual time required, the rate of heat input and the product temperatures that may be 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. Therefore, excipients often are added to increase the amount of solids.

Some consider it ideal for the dried-product plug to occupy essentially the same volume as that of the original solution. To achieve this, the solids content of the original product must be between approximately 5 and 25%. Among the substances found most useful for this purpose, usually as a combination, are sodium or potassium phosphates, citric acid, tartaric acid, gelatin and carbohydrates such as dextrose, mannitol and dextran.

Each of these substances contributes appearance characteristics of the plug, such as whether dull and spongy or sparkling and crystalline, firm or friable, expanded or shrunken 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 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 dry solid. This may be desirable when large volumes of a product are processed, but the risk of contamination from the environment is high.

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.

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Fig 27. Aseptic loading of freeze-drier (courtesy, Upjohn).

Available freeze-driers (Suppliers: *Edwards, Finn-Aqua, ETS, Hull, NRC, Stokes, Virtis*) range in size from small laboratory units to large industrial models such as the one shown in Fig 27. Their selection requires consideration of such factors as

The tray area required.
The volume of water to be removed.
Whether or not aseptic processing will be involved.
Is internal stoppering required?
Will separate freezers be used for initial freezing of the product.
The degree of automatic operation desired.

Other factors involved in the selection and use of equipment are considered in the literature.[25]

Freeze-drying is now being used for research in the preservation of human tissue and is finding increasing application in the food industry. Progress on new developments is being made in both the process and the equipment.[26]

Quality Assurance and Control

The importance of undertaking every possible means to assure 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 that 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 assuring quality of a product prospectively. QC embodies the carrying out of these plans during production and includes all of the tests and evaluations performed to be sure that quality has been achieved 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 39. During the discussion of the preparation of injections, mention was made of numerous quality requirements for components and manufacturing processes. Here, only selected tests characteristically required before a finished product is released will be discussed briefly, including sterility, pyrogen and particulate tests.

Sterility Test

All lots of injections in their final containers must be tested for sterility. The USP prescribes the requirements for this test for official injections. The FDA uses these requirements as a guide for testing unofficial sterile products. The official test has acknowledged limitations in the information that it can provide, particularly those limitations inherent in microbiological procedures. Therefore, it should be noted that this test is not intended as a thoroughly evaluative test for a product subjected to a sterilization method of unknown effectiveness. It is intended primarily 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 84.

In the event of a sterility-test failure, the immediate issue concerns whether the growth observed came from viable microorganisms in the product (true contamination) or from adventitious contamination during the testing (a false positive). The USP does permit a retest but the position of the FDA is that retest results are only valid if persuasive evidence exists that the cause of the initial sterility-test failure resides in the laboratory. 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 that group of product containers which has been subjected to the same sterilization procedure. For containers of a product which 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 when there was no change in the filling assembly or equipment and which is no longer than one working day or shift.

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 30. Rabbits are used as test animals in Section ⟨151⟩ because they show a physiological response to pyrogenic substances similar to that by man. While a minimum pyrogenic dose (MPD), the amount just sufficient to cause a positive USP Pyrogen Test response, sometimes 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* 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 Amebocyte 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.[27] 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 also has been automated.[28]

The LAL test is a semiquantitative test. To provide standardization for the test, the USP has established a reference endotoxin against which lots of the lysate are standardized.

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Thus, the sensitivity of the lysate is given in terms of endotoxin units (EU). Most USP injections now have been given limits in terms of EUs (eg, Bacteriostatic Sodium Chloride Injection, 1.0 EU/mL) as another measure of the purity of the product.

Particulate Evaluation

Particulate matter in parenteral solutions long 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. While data defining the extent of risk and the effects produced still are 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.[29] Further, it has been shown that the development of infusion-phlebitis may be related to the presence of particulate matter in intravenous fluids.[30]

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 that each final container of an injection be subjected individually to a visual inspection and that 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 which contain particulate matter that may be harmful psychologically or organically to the participant. Therefore, all of the product units from a production line currently are 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 which normally contain visible particles. Slow progress has been made on developing equipment for automated inspection of product containers.

Since it is recognized that visual inspection will not detect the presence of particles smaller than approximately 50 μm in size, the USP has established a microscopic test method for identifying particles in large-volume intravenous solutions and has set limits of not more than 50 particles/mL of 10 μm and larger in size and not more than 5 particles/mL of 25 μm and larger in size. This method consists essentially 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 oblique light, under a microscope, at both 40x and 100x magnification. These standards are being met readily by the large-volume parenteral solutions currently being manufactured in the US.

More recently the USP established standards for small-volume parenterals to be given intravenously, using an electronic instrument that counts and measures the size of particles by means of a shadow cast by the particle as it passes through a high-intensity light beam (Suppliers: *Climet, HIAC*). The limits prescribed are not more than 10,000 particles/container of ≥ 10 μm in size and not more than 1000 particles/container ≥ 25 μm in size. These specifications were developed on the premise that as many as five such products may be added to a 1-L bottle of a large-volume parenteral and five products should not contribute more than the overall limits of particles prescribed for a large-volume parenteral. Whether or not these standards are realistic toxicologically has not been established; rather, the objective of the compendium is to establish specification limits that would encourage the preparation of clean parenteral solutions, particularly for those to be given intravenously.

It also should be realized that administration sets and the techniques used in the hospital for preparing and administering intravenous infusion fluid may introduce substantial amounts of particulate matter into an otherwise clean solution. Therefore, the pharmaceutical manufacturer, the administration set manufacturer, the hospital pharmacist, the nurse and the physician must share responsibilities for making sure that the patient receives a clean intravenous injection.

The USP methods for counting and sizing particulate matter in intravenous solutions are not the only methods available for such determinations. A number of electronic particle counters are available that use the light-scattering principle to count particles in a liquid sample (Suppliers: *Climet, Met One, HIAC/Royco*). There also is an instrument available which counts particles and sizes them by measuring the effect on the resistance between two electrodes as the particles pass between them (Supplier: *Coulter*). It is obvious that only the visual inspection can be used for in-line evaluation of every container produced commercially. All of these methods require very stringent ultraclean preparation techniques to assure reasonable accuracy in counting and sizing only the particles in the solution, rather than those that may have been introduced inadvertently during the sample preparation or the testing procedure. Further, these test procedures are destructive and, therefore, can be performed only on samples of the production lot. Further information may be found in a review article.[31]

Leaker Test

Ampuls 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 microorganisms or other contaminants may enter. Changes in temperature during storage cause expansion and contraction of the ampul and contents, and will accentuate intechange if a passageway exists, even if microscopic in size.

This test usually is performed by producing a negative pressure within an incompletely sealed ampul while the ampul is entirely submerged in a deeply colored dye solution. Most often, approximately 1% methylene blue solution is employed. The test may be performed by subjecting the ampuls to a vacuum in a vacuum chamber, the ampuls being submerged in a dye bath throughout the process. Another procedure frequently employed is to simply autoclave the ampuls in a dye bath. A modification of this is to remove them from the autoclave while hot and quickly submerge them in a cool bath of dye solution. 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 a leaker test because the sealing material (rubber stopper) is not rigid. Therefore, results from such a test would be meaningless. However, evacuated bottles containing a liquid may be checked for a sharp "click" sound produced when struck with an implement such as a rubber mallet or the ball of the hand. 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 Cos-

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metic 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 to provide additional assurance that the product does not have unexpected toxic properties. Safety tests in animals are discussed in detail in the USP.

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 should provide ample protection for the product against physical damage from shipping, handling and storage as well as protecting light-sensitive materials from ultraviolet radiation. An extensive review of this subject has been published.[32]

Packaging—The USP includes certain requirements for the packaging and storage of injections, as follows:

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 exampt 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 of the information needed to assure the safe and proper use of the therapeutic agent. Since all of this information cannot be placed on the immediate container and be legible, it may be provided on accompanying printed matter. General labeling requirements for drugs are discussed in Chapter 110.

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 that 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. 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.

The label must state the name of the vehicle and the proportions of each constituent, if it is a mixture; the names and proportions of all substances added to increase stability or usefulness and the expiration date where required by the individual monograph.

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

Injections intended for veterinary use are so labeled.

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