Chapter 14

Sterile Filtration, Filling, and Lyophilization of Product

Terms

This chapter provides both an opportunity to identify the purpose of filtration in pharmaceutical applications as well as an overview of typical methods used in filling pharmaceutical products, focusing on parenteral manufacturing. This chapter also provides an overview of lyophilization, or freeze-drying, which is a way to increase the shelf life of certain products that are unstable in the solution state. Freeze-drying can also be used to reduce the weight of certain materials for easier transportation. The chapter begins with a brief introduction to the principles and history of freeze-drying, followed by the different systems utilized in modern freeze-dryers. The lyophilization properties of different materials typically used; the different phases of the freeze-drying process; and how optimized lyophilization cycles are designed are all addressed in this chapter. Finally, the chapter will conclude with the manufacturing of lyophilized products and the processes used to test for residual moisture.

After completing this chapter the student will be able to:

- explain filter validation, bacterial retention, extractables, and compatibility.
- explain filter integrity testing.
- define the physical properties of different materials and how they freeze-dry.
- identify the different components that make up a modern freeze-dryer.
- discuss the different phases of a lyophilization process.
- explain the modern manufacturing process for producing freeze-dried products.
- define the importance of and understand the different ways that residual water is measured.

Objectives

***µm*:** 0.2µm equals 0.000007873992 inches (a human hair is 40 to 120 µm thick)

Amorphous systems: solids were molecules form a solid in complete disarray; the structure is held together as a highly viscous system with no bonds being formed between the molecules.

Ampoule: a sealed glass or plastic bulb containing solutions for hypodermic injection; ampoules are the least expensive form of pharmaceutical packaging; common in third world and many veterinary applications.

Aseptic: 1) without (free of) disease-causing microorganisms 2) preventing infection; designed to prevent infection from pathogenic microorganisms

Aseptic processing: the process by which a sterile product (typically pharmaceutical) is packaged in a sterile container in a way that maintains sterility

Automated Filter Integrity Testing (AFT): an instrument that performs the typical integrity tests used in pharmaceutical manufacturing (e.g., bubble point, diffusive flow, pressure hold, and water intrusion)

Bacterial retention: validation that confirms the filter membrane's ability to produce a sterile effluent when exposed to a pharmaceutical product with 107 CFU of B. diminuta per cm^2

Capacitance manometer: a gauge that measures vacuum; this gauge senses changes in pressure by using a fluctuating metal diaphragm; this device is a capacitor, and the capacitance is directly proportional to the dielectric current of the medium relative to that of air and to a geometry factor; in normal operation of a capacitor, the geometry factor is held constant, and the dielectric constant is varied; for the purpose of freeze-drying the opposite is true; the thin metal diaphragm is sealed between two fixed electrodes; the reference side of the device is sealed and a small vacuum is created; the other side is open to the lyophilization chamber; as a vacuum is created in the freeze-dryer, the diaphragm stretches (changes geometry), and the capacitance change results in a change in voltage, which is correlated back to pressure on the readout; these types of sensors are not thrown off of set point by water vapor like the Pirani gauges, so they are reading accurate vacuum values during the entire cycle regardless of the type of gas present.

Colony Forming Unit (CFU): an estimate of microbial numbers (microbial cells rarely exist as single cells—e.g., streptococcus-chains, staphylococcus-clumps) forming a discrete colony

Container/closure system: refers to the sum of packaging components that together contain and protect the drug dosage form

Crystalline systems: solids where molecules line up in a precise pattern known as a crystalline lattice; chemical bonds and other attractive forces hold the structure together.

Differential Scanning Calorimetry (DSC): an analytical instrument used to determine, among other things, the eutectic melting and glass transition temperatures of a frozen solid

Eutectic: two or more crystalline species in such close contact that they melt as a single, pure substance

Eutectic melt: the characteristic temperature where a eutectic solid melts

Flexible IV (intravenous) bags: a sterile bag used to hold fluids that are administered to a patient intravenously (in the veins); these fluids can be anything from mixtures of medicines, to blood for a blood transfusion, to saline for hydration.

Freeze-drying (lyophilization): the process of removing water, or other solvent, from a frozen matrix by converting the water directly from a solid phase (ice) to a vapor phase under high vacuum through a process known as sublimation

Glass transition temperature: the temperature where a solid, glassy phase begins to melt

Glassy phase: an amorphous solid

Hydrophilic: water loving; spontaneously wets when exposed to water.

Hydrophobic: water hating; does not spontaneously wet when exposed to water.

Integrity testing: a non-destructive method of checking for defects in a filter membrane; an integral filter has the ability of removing bacteria from a solution (linked to bacterial retention).

Karl Fischer: a method used to test for residual moisture (volumetric or coulometric)

Metastable systems: a solid phase that formed as an amorphous phase on freezing when it should have formed a eutectic phase

Near Infrared Spectroscopy (NIR): the wavelengths of electromagnetic radiation between 800 and 2500 nm; an alternative technique used to measure residual moisture in lyophilized products.

Parenteral: not through the alimentary canal but rather by injection through some other route, such as subcutaneous or intramuscular

Pirani gauge: a gauge designed to measure vacuum; a wire in the gauge is heated to a certain temperature and exposed to a vacuum in a chamber; at atmospheric pressure there are many molecules in the ambient air that strike the surface of the heated wire and carry away some heat; it requires more energy to keep the wire heated to the same temperature at atmospheric pressure; under vacuum many of the molecules that would carry heat away from the hot wire are removed, and the system has to supply less energy to the wire to keep it hot; the amount of energy supplied to the wire is correlated back to vacuum in the chamber; the Pirani gauge is older technology and the reading is thrown off by water vapor, which is the main component in the chamber during the first phase of freeze-drying; most commercial freeze-dryers do not use this gauge; however, it is a valuable tool for development scientists when designing lyophilization cycles.

Pre-filled cartridge: a device of varied configuration and composition used with a syringe or auto-injector for the application of pharmaceutical material or anesthetic to a patient; often used for the self-administration of medicine (e.g., epi-pen).

Pre-filled syringes: a pharmaceutical device that eliminates the need to draw up medicine into a syringe and thus improves the speed at which certain medications can be administered; it can also increase accuracy in dosing since medicine in the prefilled syringe is expected to be the same with each dose.

Qualification: testing performed on equipment to ensure that a system is doing what it is designed to do and operating within its acceptable parameters

Thermocouple: two wires of different materials welded together at the tip that are used to measure temperature (at the tip only—point sensing); a current is placed across the wires, and the resistance to current flow is a function of the temperature of the welded tip of the wires.

Validation: testing performed on testing methods to ensure that the method is performing as intended with accuracy, precision, and specificity

Vapor pressure: the pressure a substance generates when sealed into a closed system due to molecules escaping from the surface and going into the headspace

Vial (SVP -<100mLs, LVP> 100mL):a small container, usually glass, for holding liquids (e.g., a vial of medicine)

Introduction

Filtration through a membrane or bulk pad is used in pharmaceutical manufacturing to remove unwanted material from the formulated bulk solution. The primary purpose of filtration is to create a sterile final product. Sterilization by filtration is referred to as the cold method of sterilization since it is the only method that does not rely on either elevated temperature or some other form of energy (gamma irradiation, E-beam, etc.) to destroy microorganisms. Sterile filtration does not destroy microbial life; rather, sterile filtration removes or separates microbial life from the rest of the product.

During the filling of containers with a product, the most stringent requirements must be exercised to prevent contamination (especially microbial), particularly if the product has been sterilized by filtration and will not be sterilized in the final container (terminal sterilization—regulatory guidance requires that terminal sterilization be performed if the product can tolerate it; the manufacturer must prove that post-fill autoclaving does not adversely affect product stability, such as the case with most biopharmaceuticals). This process is referred to as an *aseptic fill* and is supported with media fills, cGMP facility qualification, and robust quality systems. The assurance of product sterility is supported by the design of the filling complex, the training of the operators, environmental monitoring, the performance of filter validation, and the mimicking of the manufacturing process through media fills. During the filling operation, the product must be transferred from a bulk container or tank and subdivided into dose containers (pharmaceutical container/closure). This operation exposes the sterile product to the environment, equipment, and manipulative technique of the operators until it can be sealed in the dose container; therefore, this operation is performed with a minimum exposure time, even though maximum protection is provided by filling under a blanket of HEPA-filtered laminar-flow air within the aseptic area.

Freeze-drying (lyophilization) is a process employed to remove water or other solvents from products at low temperatures. This is accomplished through a process referred to as sublimation, or a change of phase from solid to vapor without passing through the liquid state. Freeze-drying is performed mainly to increase the shelf life of products that degrade quickly in solution; however, there are industries that use this process to reduce the weight of their products for easier and/or more cost-effective transportation. For example, the National Aeronautics and Space Administration (NASA) will regularly freeze-dry food to be carried into space in order to minimize the weight and therefore minimize the cost of transportation into space. Once the products are freeze-dried they can be brought back to a usable condition by adding water or some other appropriate solvent system prior to use. Some of the products of freeze-drying are:

- injectable drug products
- diagnostic testing kits
- foods such as coffee and fruits
- fast dissolving oral tablets
- human and animal tissues

Filtration

Types of filters

There are four primary types of filters used in the parenteral and biopharmaceutical industry (Table 14-1).

Table 14-1. General types of filters used in the sterile product industry

Filter Type	Size Range (microns)	Examples of What Is Removed by This Filter Type
clarifying (particle) filters	10 to 200	pollens particles some bacteria
microfilter	0.1 to 10	all bacteria yeasts colloids
ultrafilter	0.001 to 0.1	most viruses large organic compounds (> 10,000 Daltons)
nanofilter (reverse osmosis)	< 0.001	small organic compounds ions

Clarifying filters

The porosity of particle filters ranges from 10 μm to 200μm, and they are used as depth or pre-filters to remove dirt, pollen, some bacteria, and most particles. Examples of materials used as depth filters include cellulose fibers, diatomaceous earth, glass fibers, sand and gravel, and polypropylene yarn. Depth filters are extremely efficient at removing "dirt" from solutions. Pre-filters or surface filters are commonly used simply to protect the membrane microfilter from clogging too quickly. Examples of materials used as pre-filters include cellulose ester and heat bonded polypropylene fibers.

Microfilters

Microfilters are the sterilizing filters used in the industry. These filters have relatively narrow pore distribution because of controlled polymeric structures and can be integrity tested. Examples of materials removed by microfilters are listed in Table 14-1.

The porosity of microfilters ranges from 0.1μm to 10 μm, and they are used to remove all bacteria, yeast, and colloidal forms. Combination filters also exist, which combine either various membrane pore sizes or depth media and a membrane filter. Microfilters have a rated porosity of 0.2 μm or smaller. Filters can be rated either nominally or absolutely. Filter manufacturers assign arbitrary micron values based on data obtained in the removal of samples of known particle sizes at different weights. For example, a filter having a nominal rating of 67 percent for one micron or greater means that such a filter will remove 67

percent of all particles $\geq$ 1 μm. Nominal ratings describe the weight percent removal of particles/bacteria at a particular size. Absolute ratings, much more commonly used in the sterile filtration industry, define the diameter of the largest particle that will pass through the filter; therefore, unless the filter is damaged it will retain particles 0.2 μm and larger. Typically the formulated product must be filtered prior to being filled in its final container/closure. Removing particulate matter by filtration through a 0.2 μm membrane will eliminate microorganisms and sterilize the product.

Ultrafilters

The porosity of ultrafilters ranges from 0.001 μm to 0.1 μm, and they are used to remove most viral life forms and large organic compounds (> 10,000 Daltons—one Dalton = 1.66×10^{-27} kg abbreviated). Ultrafilters are often used in upstream manufacturing for cell harvesting and buffer exchange.

Nanofilters

The porosity of nanofilters is less than 0.001 μm, and they are used to remove small organic compounds and ionic forms. These filters are used in reverse osmosis systems.

Polymeric filter materials are broadly classified as either hydrophilic or hydrophobic. Hydrophilic filters wet spontaneously and are used in sterile filtration of aqueous solutions. Hydrophobic filters do not wet spontaneously and are used in the sterile filtration of gases, solvents, or strongly acidic or alkaline solutions.

Mechanisms for removal of particles and microorganisms

Filters are thought to function by one or, usually, a combination of the following: sieving or screening[1], entrapment or impaction[2], and electrostatic attraction-adsorption[3] (Figure 14-1).

[1] Meltzer, TH, Jornitz, MW, Johnston, PR, Relative efficiencies of double filters or tighter filters for small-organism removals, Pharm. Tech., 1999, 23, 98-106.
[2] Sundaram, S, Eisenhuth, J, Howard Jr., G, Brandwein, Retention of water-borne bacteria by membrane filters, Part I. Bacterial challenge tests on 0.2 and 0.22 micron rated filters, PDA J Pharm Sci Tech, 2001, 55,65-86.
[3] Sundaram, S, Mallick, S., Eisenhuth, J, Howard Jr., G, Brandwein, Retention of water-borne bacteria by membrane filters, Part II. Scanning electron microscopy (SEM) and fatty acid ester (FAME) characterization of bacterial species recovered downstream of 0.2/0.22 micron rated filters, PDA J Pharm Sci Tech, 2001, 55, 87-113.

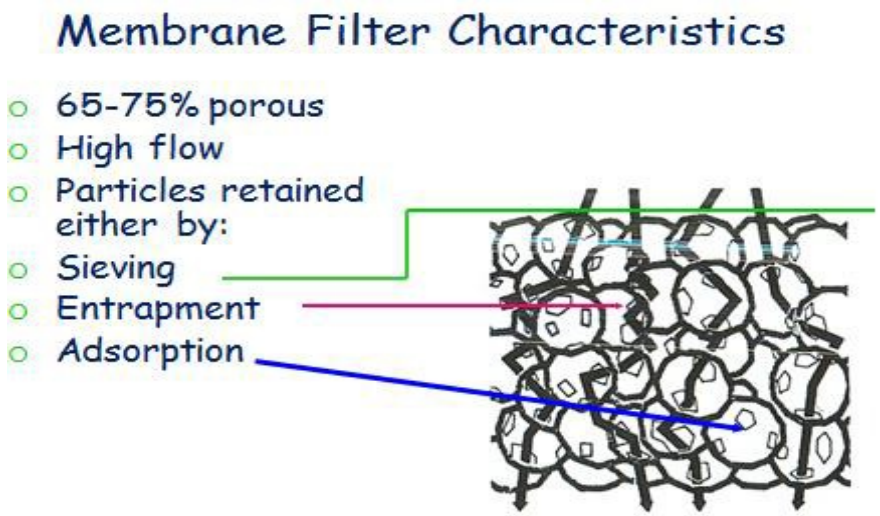


Figure 14-1. Membrane filter characteristics (courtesy EMD Millipore Corporation)

When a filter retains particles by sieving, they are retained on the surface of the filter. Filters become more efficient at removing particles as the layer of filtered particles (cake) on the surface becomes thicker. 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 that are opposite in charge to that of the surface of the filter pore to be held or adsorbed to the surface. 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.

Factors affecting filter efficiency in microbial and particle retention[4]

- Type of particle–source, shape, charge, and size
- Filter material–filter composition plays a role in charge-related attraction of particles.
- Filter membrane thickness–filter thickness slows the flowcharacteristics and affects the particle adsorption mechanism; Although a coarse, thick membrane can be just as efficient as a fine, thin one, it may reduce the flow rate.
- Filter porosity–the smaller the porosity, the greater the retention of microorganisms and particles, but there is greater resistance to liquid moving through the membrane, and flow rates are retarded. Also, potential incompatibilities with liquids being filtered are greater with smaller porosities—the smaller the pore, the greater pressure needed for integrity testing.

[4] Meltzer, TH, Jornitz, MW, Johnston, PR, Relative efficiencies of double filters or tighter filters for small-organism removals, Pharm. Tech., 1999, 23, 98-106.

- Temperature–influences microbial growth and affects the surface tension of the liquid being filtered.
- Type of fluid/solution being filtered–increasing the viscosity of the solution will require some increase in applied pressure that, in turn, will increase the shear force on any bacterial cells present. Increased viscosity will disrupt adsorption interactions on the filter membrane but will have no effect on size exclusion properties of the membrane. Surface-active agents (Polysorbate 20/Polysorbate 80) in the solution formulation will decrease the surface tension of the solution and lower the bubble point of the filter (explained later). Surface-active agents will normally bind to solid surfaces and may reduce or eliminate bacterial adsorption in the filter but will have no effect on either membrane structure or size of the bacterial cell. Bacterial cells in a hypertonic solution may experience crenation (loss of water in the cell due to osmosis) that potentially may allow migration through a filter membrane that would otherwise not allow the passage of the organism under isotonic conditions.
- Applied pressure, flow rate, and time–when filters are used with the validated time intervals and within the manufacturer's operational specifications they are not affected by increasing pressure or flow rate or time. Membrane sterilizing filters can be used for an extended period of time without observing changes in retention characteristics according to filter manufacturer's technical literature. Filters used for sterilizing gases may be in place for months at a time.

All filter manufacturers have innumerous technical articles and data sheets (most available on their websites) describing and explaining the properties and functionalities of all their commercially-available filters.

Applications

Membrane filters are used exclusively for sterilizing solutions because of their particle-retention effectiveness, non-shedding property, non-reactivity, and disposable characteristics. However, it should be noted that non-reactivity does not apply in all cases. For example, polypeptide products may show considerable adsorption through some membrane filters, but those composed of polyethersulfone (PES) and polyvinylidine difluoride (PVDF) have been developed to be essentially non-adsorptive for these products. The most common membranes are composed of cellulose esters, nylon, polysulfone, PES, PVDF, or polytetrafluoroethylene (Teflon) (Table 14-2).

Table 14-1. Microfilter and ultrafilter polymers[5]

Membrane polymer	Advantages	Disadvantages
cellulose acetate	extremely low adsorption high flow rates	limited pH compatibility
cellulose nitrate	good flow rate	high adsorption limited pH compatibility

[5] Jornitz MW, Filters and Filtration, Encyclopedia of Pharmaceutical Technology, Informa, London, 2003, p 1754.

regenerated cellulose	extremely low adsorption extremely high flow rates	limited pH compatibility
modified regenerated cellulose	extremely low adsorption broad pH compatibility	moderate flow rates
polyamide (nylon)	good solvent compatibility good mechanical strength broad pH compatibility	high protein adsorption moderate flow rates
polycarbonate	good chemical compatibility	moderate flow rates difficult to produce
polyethersulfone	high flow rates broad pH compatibility	moderate-to-low adsorption limited solvent compatibility
polysulfone	high flow rates broad pH compatibility	moderate-to-high adsorption limited solvent compatibility
polypropylene	excellent chemical and mechanical resistance	hydrophobic material high adsorption
polyvinylidene difluoride (PVF)	low adsorption good solvent compatibility	moderate flow rate hydrophobic base; made hydrophilic by chemical surface treatment high cost
polytetrafluoroethylene (PTFE)	excellent chemical and mechanical resistance	hydrophobic material high adsorption high cost

Filters can be either flat membranes (stacked disk filter such at Millipore's Millipak® system) or pleated into cylinders or cartridge filters (Figure 14-2) to increase surface area and, thus, increase flow rate (suppliers include Cuno, Meissner, Millipore, Pall, and Sartorius).

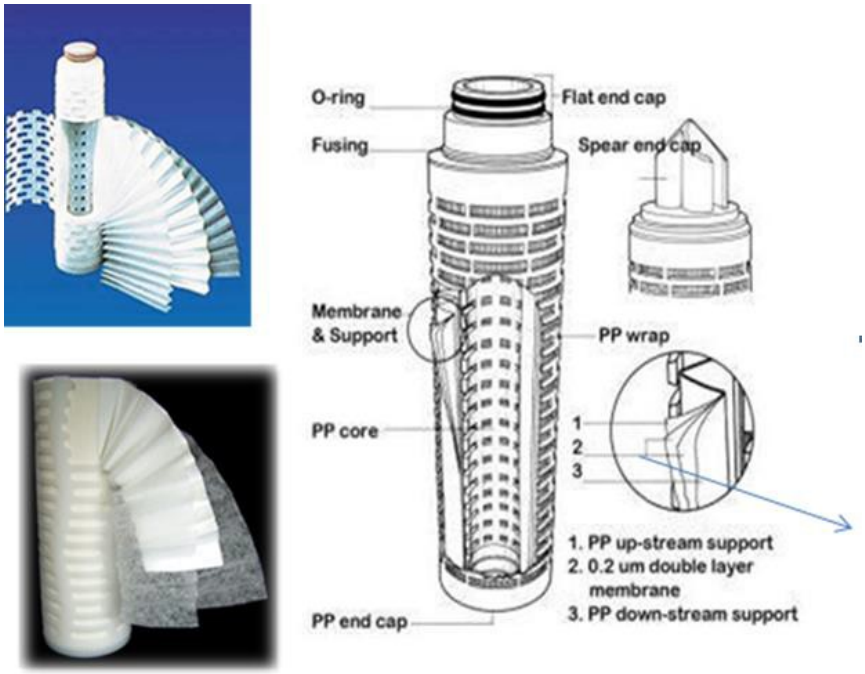


Figure 14-2. Cylinder or cartridge filters (courtesy EMD Millipore Corporation)

Capsule filters are cartridges encapsulated in a rigid shell (usually polycarbonate or polypropylene) and are completely disposable, as they do not require stainless steel housing for use. Fluid enters the outside of the filter capsule/cartridge with applied positive pressure forcing the fluid inward through the filter with the sterile effluent exiting from the center of the filter (Figure 14-3).

Figure 14-3. Capsule filters
(courtesy EMD Millipore Corporation)

When using a cartridge, the filter is assembled in the stainless steel housing by wetting the O-rings; making certain that the filter is oriented properly within the housing; and then hand tightening the clamps. Care must be taken to avoid damaging the filter membrane during installation. The filter and housing are then steam sterilized, typically by autoclaving the assembly. Filters in stainless steel housing may be sterilized by steam-in-place (SIP) systems. Inlet pressure must be matched to the maximum cartridge temperature with differential pressure controlled to ensure filter integrity. Both pressurization before and depressurization after sterilization must be gradual; the quick vacuum pulses in a modern autoclave may create a pressure differential in a redundant system that may result in capsule filters becoming out of round.

In SIP applications, care must be taken that air is replaced by steam, and any condensate must be removed from the system to allow proper sterilization. For stacked disk filters, care must be taken to ensure that the possibility of reverse-flow pressure is prevented, as this type of filter cannot tolerate more than a few pounds per square inch gage (psig), especially at temperatures over 80ºC. Validation of the sterilization of filters occurs with thermocouples and spore strips located at identified cold spots within the filter assembly. After sterilization, the liquid process filter cartridges may be dried with filtered compressed gas; filters used in gas purification applications must be dried prior to use, as any liquid remaining on the membrane may reduce or eliminate (blind) the ability of the filter to allow the flow of gas through the membrane.

Filter validation

Filter validation includes both destructive testing to qualify the filter initially and non-destructive testing that is performed prior to and after using the filter in batch production. Destructive testing includes three main tests—(1) bacterial retention using actual final formulation of drug product, (2) filter extractables/leachables (most often a model solvents approach is applied), and (3) compatibility of filter with drug product.

Bacterial Retention

In this testing phase the filter is challenged with a known population of microorganisms using conditions that simulate the actual process. It is important that the microbial challenge involves the final product formulation. Formerly it was acceptable to use a placebo form of the final product, where critical attributes like pH, viscosity, osmolarity, ionic strength, and surface tension were simulated containing the microbial challenge. The antimicrobial properties of the formulation must be neutralized before adding the microbial challenge. Often the filter is exposed to the product, completely rinsed from the membrane, then a surrogate loaded with the microorganism.

The microorganisms used are *Brevundimonas diminuta*, selected because these cells are approximately 0.3 μm in size, barely above the absolute rating of the 0.2 μm sterilizing filter. The concentration of these cells is 10^7 cells per cm^2 filter surface area. Processing conditions that are simulated include the following:

- filtration pressure and flow rate
- duration of filtration process
- using the same filter type that will be used in actual process
- temperature

Changes in the formulation or process parameters may affect the filter validation and must be considered. Most filter validations are performed after the formulation is fixed and the process parameters are established. If significant changes are made in either of these, re-validation may be required.

Figure 14-4 depicts a schematic of the filter validation retention test apparatus used at filter manufacturers. Note that the apparatus uses both a 0.2 μm filter and a 0.45 μm filter with the latter used as a positive control.

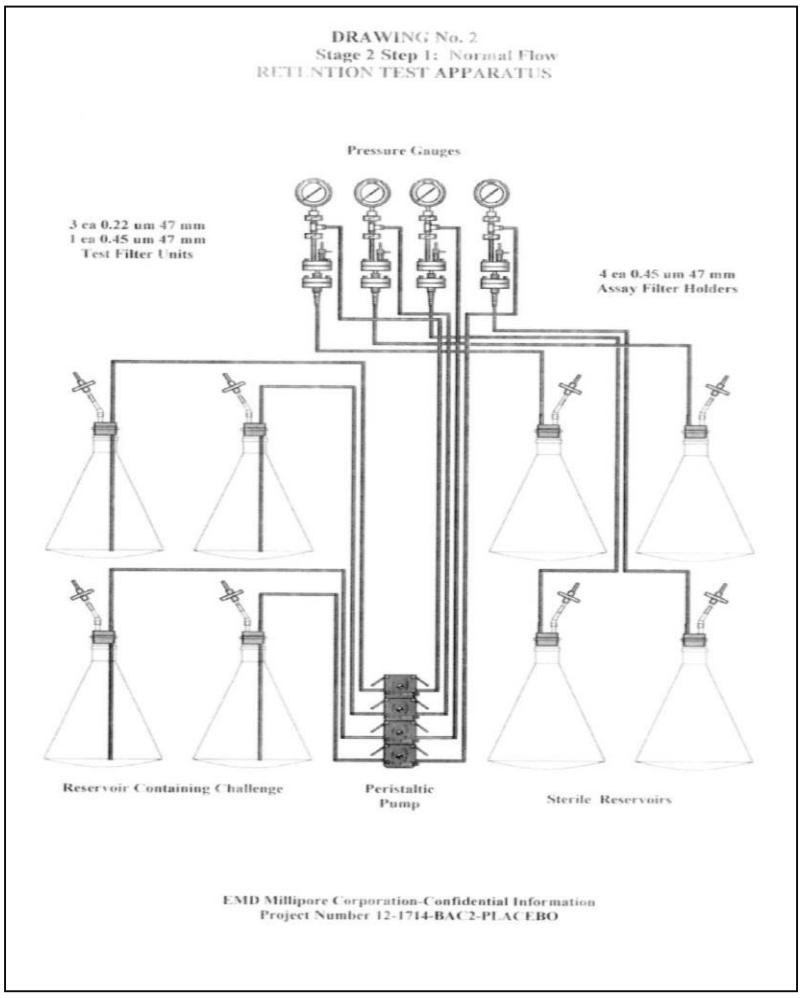


Figure 14-4. Filter validation retention test apparatus (courtesy EMD Millipore Corporation)

Product/Filter Compatibility

Tests must be performed to demonstrate that (1) the product does not adversely affect the retention properties of the filter, as is accomplished in most cases with the bacterial retention studies discussed above, and (2) does not cause the filter to excessively leach materials into the product. Every filter adds something to the product stream during use. Filter compatibility and extractable studies are performed by the filter manufacturer, although like bacterial retention studies, the product manufacturer is ultimately responsible for the validity of the data. The filter manufacturer provides both information on the flush volume required to yield negative oxidizable substances and the data on the level of extractables obtained with different model solvent exposures. Potential filter extractables include oligomers, mold release agents, antioxidants, wetting agents, manufacturing debris, plasticizers, membrane backing, cartridge body, and 0-ring material. Most of these are removed during the wetting for the pre-use integrity testing of the filter.

Studies to ensure that the filter does not adversely affect the product are typically performed by the process-owner rather that the filter manufacturer. Methods for testing product stability and the product degradants are often complex and difficult to transfer, so it is often easier to perform these tests in-house rather than contract the manufacturer for the same. Protein drug products can occasionally bind to the filter material involved in in-line membrane filters. The amount of protein lost adsorbing on the filter surface area and occupying all the binding sites may not be significant. Depending on the batch size; the surface area of membrane used; and the protein's affinity toward binding on the membrane, the product's protein concentration may be affected. A pre-flush step with a buffer surrogate containing a surfactant prior to product filtration can be performed to both occupy available binding sites and remove potential extractables. PES and PVDF filters are low protein-binding filters. Low-binding is a relative term, and actual loss should be confirmed prior to manufacture.

Other data provided by the filter manufacturer in performing qualification studies on the filter to be used with the finished product include:

- limits for flow rate, temperature, and pressure
- assurance that the filter meets the non-fiber releasing criteria from 21CFR 210.3b. (sterilizing filters from the major manufacturers all meet these criteria)
- procedures for filter sterilization
- the filter bubble point or diffusion rate for the in-process integrity tests
- correlation of the integrity test value and the amount of *B. diminuta* retained
- written instructions and specifications for the filter integrity test

In-process filter integrity testing

Prior to actual filtration of the product, the filter should be flushed with either product or water for injection to reduce potential extractables and downstream particles. The filter is then subjected to a filter integrity test (pre-filtration filter integrity test), and after the solution is filtered, the filter is again subjected to a second filter integrity test (post-filtration filter integrity test). This integrity test is usually performed either as the bubble point test or as the diffusion or forward flow test. The bubble point test is commonly used on smaller filters. As the surface area of filters becomes larger, diffusion of air through the water-filled pores tends to obscure the bubble point. Therefore, the diffusion test has been developed as an integrity test for filters with large surface areas. A *pressure hold test* can also be applied to large surface area filters. The filter manufacturer recommends the best integrity test for the filter system in question.

These are tests to detect the largest pore or other opening through the membrane. The basic test is performed by gradually raising air pressure on the upstream side of a water-wet filter. It is critical that the filter be completely wetted; if not, the integrity test will fail because applied pressure gas will easily pass through pores not filled with liquid. Either water for injection or actual product is used to wet the filter prior to performing the pre-

filtration filter integrity test. The bubble point test raises pressure until a pressure is obtained; where air bubbles first appear downstream is the bubble point.

The principle of the bubble point test is that a fully wetted membrane filter of extremely small pore size will hold liquid in the pores by surface tension and capillary force. The pressure of a gas required to force the entrapped liquid both through and out of the fully wetted pore capillary is referred to as the bubble point because air bubbles will appear after the liquid is forced out (Figure 14-5). The bubble point is a function of the type and pore size of the filter membrane, the surface tension of the liquid, and temperature.

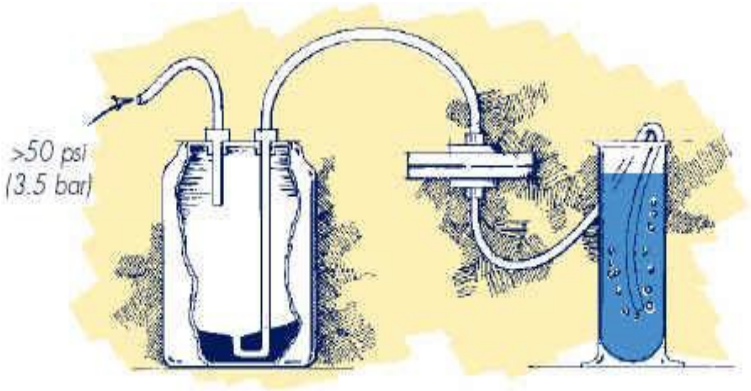


Figure 14-5. Bubble point test
(courtesy EMD Millipore Corporation)

The bubble point pressure is correlated to the microbial log reduction value as determined by the filter manufacturer, so that filter having a bubble point greater than the established specification is shown to retain B.diminuta. Table 14-3 provides the standard bubble point ratings for various types of membrane filters (although these ratings are subject to change).

Table 14-3. Examples of bubble point and diffusion test standards for filter integrity criteria of different filters (from Millipore Corporation technical literature)

Bubble points

Filter	Bubble point (≥ pounds/square inch)
0.1 µm PVDF	70
0.22 µm PVDF	50
0.2 µm MCE	55
0.45 µm MCE	30
0.65 µm MCE	17

Diffusion rates

Filter	Diffusion rate (mL/min @ X psi)
0.1 µm PVDF cartridge	< 20 @ 56
0.22 µm PVDF cartridge	< 13 @ 40
0.45 µm PVDF cartridge	< 15 @ 15

Note: membranes from different manufacturers typically have different testing standards.

The diffusion or forward flow test raises pressure to some point below the known bubble point pressure (a rule of thumb is 80 percent of the bubble point value—the manufacturer's product literature should be referenced for actual specifications) then diffusion flow (usually in mL/min) is measured. The principle of the diffusion test is similar to that of the bubble point test—a gas dissolved in a liquid held in the pores of a fully wetted filter slowly diffuses out of the filter pores as a pressure differential is applied to the filter that results in a concentration gradient across the filter. It should be understood that diffusion is always occurring when a pressure differential exists between upstream and downstream sides of the filter membrane; it is just more obvious when the upstream pressure nears the bubble point.

Figure 14-6 illustrates the set-up of a diffusion flow test apparatus, and Table 14-3 provides acceptance criteria for diffusion flow as a function of the type of cartridge filter. These pressures are characteristic for each pore size of a filter and are provided by the filter manufacturer. If the filter is wetted with other liquids, such as a product, the bubble point may differ and must be determined experimentally. Improper wetting and excessive temperature differentials between the wetting solution temperature range specifications by the manufacturer may give false failing results. If the bubble point is lower than the rated pressure, the filter may be defective, perhaps due to a puncture or tear, and should not be used.

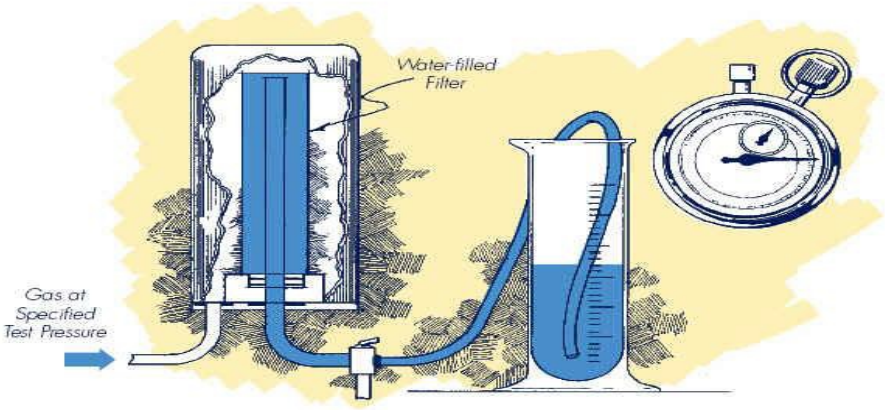


Figure 14-6. Diffusion flow test apparatus (courtesy EMD Millipore Corporation)

The manual bubble point test is best or small filters, while the manual diffusive flow test is best for large cartridge filters. However, the use of automated filter integrity testing (discussed below), with its high sensitivity to flow changes, makes filter size irrelevant to the test method.

The bubble point test is both quick and simple and relates to the largest pore size of the filter, but errors in estimating the bubble point occur, especially when manually performed. The diffusion test confirms the absence of large pores, but is not applicable for small area filters because of insufficient diffusive gas flow. Neither test measures pore size, but an acceptable result indicates that the filter is undamaged and acceptable for use. Filter manufacturers typically perform microbial retention as a lot release test so that every lot of filters can be shown to be retentive.

While membrane filter cartridges are disposable and thus discarded after use, the housings must be cleaned thoroughly between uses. Using a capsule filter eliminates the need for a housing in many filtration applications; however, most steam-in-place applications require housings. It is possible to purchase clean, sterile, pretested, ready-to-use, disposable assemblies for both small and large volumes of solutions. The use of pre-sterilized filters in disposable process assemblies is becoming more prevalent in the industry.

Membrane filter integrity testing with product that lowers the bubble point

Surface-active agents are amphiphilic wetting agents (molecules having a polar water-soluble group attached to a water-insoluble hydrocarbon chain) that lower the surface tension of water and are positively adsorbed at the liquid/air interface, thus preventing proteins from adsorbing at this interface and minimizing protein denaturation (aggregation) due to hydrophobic (air) interactions. Thus the presence of surface-active agents in aqueous solutions depresses the bubble point compared to water alone (the amount of bubble point depression is affected by the concentration of the surfactant in the formulation).

Occasionally the depressed bubble point can be restored by aggressively rinsing the filter with water; however, this is not always successful. If the surfactant cannot be easily removed from the filter membrane, filter validation studies should be conducted using the final product formulation containing the required 10^7 organisms (*B. diminuta)* per cm^2 filter surface area. Filter manufacturers, however, strongly suggest supplementing the data from the initial bubble point suppression validation with an annual review of batch bubble point values to capture any change in the process that may affect the product bubble point and require a revision of the specification.

Figure 14-7. Millipore Integritest 4 AFIT
(courtesy EMD Millipore Corporation—Integritest is a registered trademark of Merck KGaA)

Automated filter integrity testing

Current trends in GMP have moved away from manual filter testing and have encouraged pharmaceutical sterilizing filters to be pre- and post-tested using an automated filter integrity testing instrument (e.g., Millipore's IT4, Pall's Flowstar 4, and the Sartorius's SartoCheck4). The automated filter integrity tester (AFIT) is intended to perform all of the standard filter integrity tests, such as bubble point, diffusive flow, pressure hold, and water intrusion. Technology and system algorithms vary between system manufacturers, but all systems work by taking a wetted filter, applying gas pressure on the upstream side of the membrane, and measuring the change at that pressure, whether the change is in flow (Flowstar 4®) or pressure (IT4).

For diffusion type tests, the pressure on the membrane is kept constant, and the change is measured as mL/minute. An acceptable test has a flow ≤ the manufacturer's filter specification. Pressure hold and water intrusion type tests pressurize the upstream of a filter membrane with gas (oxygen or nitrogen as defined in the manufacturer's specifications); the gas is held for a

specified period of time, and the change in pressure (if any) is compared against the filter specification.

Bubble point testing is a bit more dynamic, as the pressure is incrementally increased from the diffusion test pressure (usually 80 percent of the BP specification) and the increase of flow at each change is captured and plotted by the integrity tester. The test proceeds until the pressure sufficient to create mass flow of gas through the filter membrane is exceeded. The flow/pressure at each test point is processed using the integrity tester manufacturer's proprietary algorithm and the bubble point (mass flow through the largest pores for the filter) is calculated.

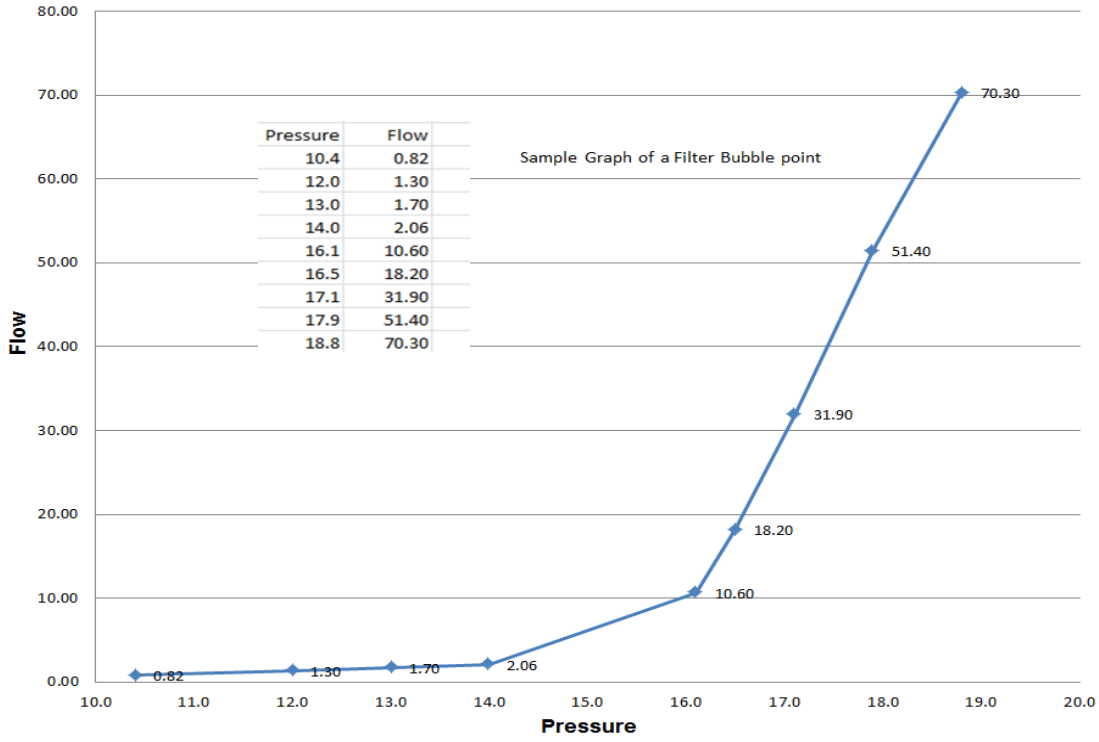


Figure 14-8. A filter bubble point

Automated filter integrity testing has numerous advantages, including elimination of operator-to-operator variability; increased sensitivity (the bubble point of a large filter can easily be determined using AFIT, while it would be impossible to manually detect mass flow of gas [bubble point] while the normal diffusive flow of the large membrane surface area is ongoing); and provision of both improved testing consistency and reproducible results.

INTEGRITEST 4

Instrument Serial Number:	IT4N0421	**Test Name:**	Method 11(P) PTFE
Date:	2013/03/19	**Test Revision:**	7
Time:	17:27:54	**Test Description:**	Acro 50 Disk vent filter 5-409
Test Serial Number:	20130319172754	**Page:**	1 of 2

Bubble Point Test

Filter Catalog Number:	6074270	**Operator Inputs:**	
Test Fluid:	70/30 IPA/WFI	Operator:	smithb19
Filter Description:	'Phobic vent	Batch:	921684
Filter Size:	50mm	Lot Number:	20016
Pore Size:	0.2	Comment:	RV#1
Housing Type:	Capsules		
Test Flags:	Skip Calibration		

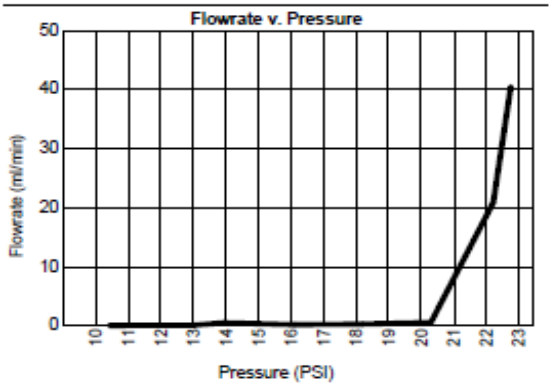
Test Specification

Diffusion Pressure:	10.4 PSI
Gross Leak:	1 ml/min
# of Cartridges:	1
Minimum Bubble Point:	13.0 PSI
Maximum Bubble Point:	25.0 PSI
Sizing Stabilization Time:	50 sec
Diffusion Stabilization Time:	50 sec

Test Data

Gas Volume: 59 ml

Pressure	Flowrate
PSI	ml/min
10.4	0.1
12.0	0.1
13.0	0.1
14.0	0.5
16.1	0.2
18.2	0.3
20.3	0.6
22.2	20.9
22.8	40.5



Test Results

Measured Bubble Point: 21.7 PSI

Test Conclusion

PASSED

Figure 14-9. Millipore Integritest 4 AFIT
(courtesy EMD Millipore Corporation—Integritest is a registered trademark of Merck KGaA)

Ongoing controversies

Occasionally evidence is reported that 0.2 µm filters do not remove all possible microbial contamination (mycoplasma, viruses) (2, 3), potentially necessitating the need to use certain types of 0.1 µm membrane filters[6]; this, however, is of greater concern in upstream manufacturing where the pharmaceutical API is manufactured via cell culture. Though most of the parenteral pharmaceutical industry continues to use 0.2 µm filters, redundant (two 0.2 µm filters in-line) filtration systems are now employed. Double filtration indeed increases the probability of adsorptive capture of organisms such as L-forms smaller than 0.2 µm filter pore size[7]. Pre- and post-filtration integrity tests are performed on both filters. It should be noted that microbial retention validation is typically performed using one sterilizing membrane.

Ongoing technical issues or controversies in sterile filtration technology include the following:

- defining worst case conditions for filter validation studies
- the need for validating the removal of the smallest types of organisms (e.g., mycoplasma and viruses [typically this is a greater concern during upstream manufacturing])
- effects of the filter and the process of bacterial deformation

Sterile Product Filling Operation

The compounded product is usually in the form of a liquid; however, products are also compounded as suspensions, emulsions, and powders. A liquid is more readily subdivided uniformly and introduced into a container having a narrow mouth than is a solid. Mobile liquids are considerably easier to transfer and fill than viscous, sticky liquids, which require heavy-duty machinery for rapid production filling.

Although numerous devices are available for filling containers with liquids, certain characteristics are fundamental to all of them. All provide the ability to repetitively force a measured volume of liquid through the orifice of a delivery tube that is introduced into the container. The size of the delivery tube ranges from a 20-gauge hypodermic needle to a tube one half-inch or more in diameter. The required size 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 both reduce the resistance and decrease the velocity of flow of the liquid. Product surface tension, viscosity, and temperature dictate the potential of product dripping or the formation of threads of product on

[6] Sundaram, S, Eisenhuth, J, Howard Jr., G, Brandwein, Retention of water-borne bacteria by membrane filters, Part III. Bacterial challenge tests on 0.1 micron rated filters, PDA J Pharm Sci Tech, 2001, 55, 114-126.
[7] Jornitz, MW and Meltzer, TH, Sterile double filtration, Pharm Tech., 1998, 22, 92-100.

the sealing surface of the vial or syringe wall. To reduce the possibility of the product splashing out of the container, most automated filling systems fill “bottom up,” with the filling tube inserted to its greatest depth at the start of the filling cycle and withdrawing as the product is dosed into the container.

For smaller volumes of mobile liquids, the delivery is usually obtained from the stroke of the plunger of a syringe or rotation of a peristaltic pump, forcing the liquid through a delivery tube/needle combination and into the final container /closure system. For heavy, viscous liquids, a sliding piston, the turn of an auger in the neck of a funnel, or the oscillation of a rubber diaphragm may be used. For large volumes the quantity delivered is usually measured in the container by the level of fill in the container; the force required to transfer the liquid being provided by gravity; a pressure pump; or a vacuum pump.

The narrow neck of an ampoule limits the clearance between the delivery tube and the inside of the neck. Since a drop of liquid normally hangs at the tip of the delivery tube after a delivery, the neck of an ampoule is 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 backinto the lumen of the tube—a suck-back feature. Since the liquid is in intimate contact with the parts of the machine through which it flows, these mechanisms must be constructed of non-reactive materials, such as borosilicate glass or stainless steel. Modern coatings, such as Advanced Materials Component s Express AMCX2286, are used to coat stainless steel needles for products that are affected by contact with metals (e.g., formulations containing chelating agents or those having extremely acidic or alkaline pH values). Additionally, these mechanisms should be easily demountable for cleaning and sterilization. A growing trend in the industry is to use completely disposable product contact equipment, eliminating the need for post-use cleaning.

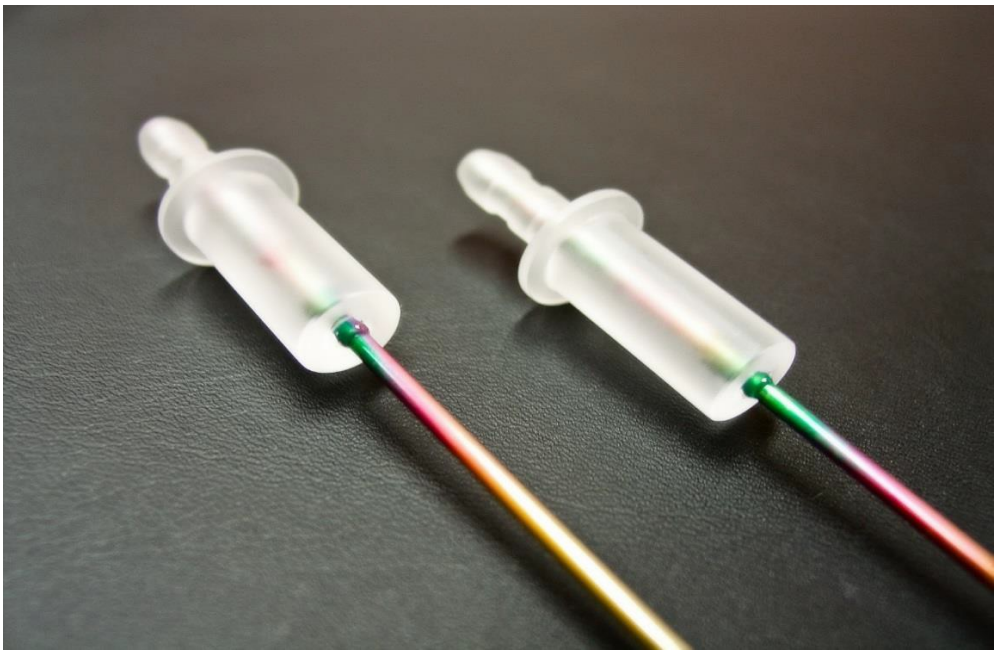


Figure 14-10. Coated disposable filling needles (courtesy Overlook Industries)

Figure 14-11. ATMI mag-mixing systems (courtesy ATMI Life Sciences)

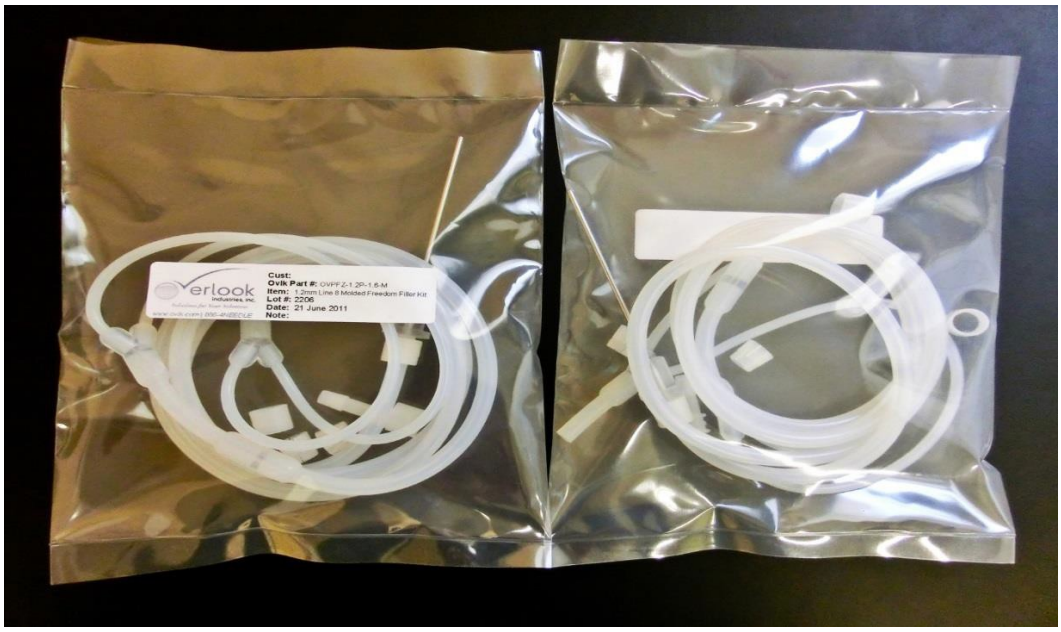


Figure 14-12. Freedom filler kit disposable filling system (courtesy Overlook Industries)

Filling mechanisms

Filling machines are classified by the type of driving device or filling mechanism used to deliver the drug-containing formulation into the primary package. There are at least four driving devices and four filling mechanisms:

Driving Device	Filling Mechanism(s)
gravity (solids and liquids)	gravimetric, time pressure, fill by weight
piston (liquids and gases)	rotary piston, rolling diaphragm pump
rotary pump (liquids and gases)	rotary peristaltic
auger screw or vibrator (solids)	vibratory/mechanical force

Possible Problems Encountered During Filling Operations

- product splashing
- product spills
- product foaming and effect on dose accuracy
- viscous product and potential problems with dose accuracy and uniformity
- out of tolerance fill volumes/weight
- receiving vessel overflows
- receiving vessel over pressurized
- bent filling needles
- filling needles clogged with product
- control of dose from container to container
- adsorption of active ingredient on the surface of the tubing used with the filling machine
- protein aggregation due to tubing surface interactions
- leachables from tubing
- fill pump leak
- power outage

Gravity/Time Pressure Filling

The gravity based filling machine is both the oldest and most economical type. The filling principle is simple—the amount of product flowing through the filling nozzle is driven by gravity and is always constant for a fixed amount of time. The finished bulk solution is pumped into a holding tank above a set of pneumatically operated valves. Each valve is independently timed by a master computer so that precise amounts of liquid flow by gravity into the container. The amount of product dispensed is controlled by adjusting the time for closing the valve. These systems can range from bulk bag systems working with thousands of kilograms to more precise systems in which weight feedback is used to control the volume of dispensed product. Independent timing of each filling valve/nozzle corrects for minor variations in flow rates so

that each container is filled accurately and uniformly. Improvements in holding tank headspace, pressure control, and feedback control have made time pressure filling machines more accurate than pump systems for many applications.

The disadvantage of this type of technology is that the dynamics of the fluid path and nozzle actuation characteristics continuously change over time. This requires the operator to make adjustments to the machine's stored parameters more frequently than with other filling mechanisms. Automated weight check with a consistent feedback to the filler control system reduces the number of operator interventions.

	Strengths	Weaknesses
Time pressure	▪ clean—few parts in the product path ▪ easy to maintain and change over ▪ product path easy to CIP/SIP ▪ minimal potential for leakage in product path ▪ ability to handle sensitive products ▪ ability to run dry ▪ accurate for small or large fill volumes ▪ possible self-adjustment of fill volumes between fill cycles at line speed	▪ pressure change sensitivity ▪ pressure control and monitoring required ▪ temperature sensitivity ▪ viscosity sensitivity ▪ expensive parts
Fill by weight	▪ clean ▪ easy to maintain and change over ▪ easy to clean and to CIP/SIP ▪ minimal potential for leakage in product path ▪ ability to handle sensitive products ▪ ability to run dry ▪ real time fill volume control ▪ 100 percent documentation of fill volumes ▪ accurate for small or large fill volumes	▪ high cost of scales and control system ▪ maintenance (potential for spillage on scales) ▪ complex container handling ▪ longer fill times ▪ accuracy for small fills decreases with fill volume reduction ▪ dripping or spills can damage scales—protection of scales complicates the system

[8]

Piston Filling

Piston filling includes pumps with either lapped rotary or check valves as well as those that use a rolling diaphragm. Lapped rotary pumps involve a cylinder that is lapped by both the piston

[8] Peterson, A. Filling methods as they apply to parenteral product quality and biopharmaceutical microdosing. In Practical Aseptic Processing: Fill and Finish. Vol 1, Lysfjord, J. ed, Parenteral Drug Association, Bethesda, MD, 2009, 145-165.

and the rotary valve to produce an exceedingly tight fit. Pumps with check valves are not typically used for injectable filling because the valves are difficult to clean and have a tendency to seize. Pumps with the rolling diaphragm use a flexible membrane attached to the pump at its outside diameter and to the piston at its inside diameter. A space between the piston and the internal cylinder allows the diaphragm to be doubled and to roll as the piston moves up and down. Vacuum may be used to maintain the shape of the diaphragm and pull the piston downward on the refill phase of the filling cycle.

Piston pumping machines are the most commonly used filling machines for liquids. They may not be the best choice as a filling mechanism for shear-sensitive liquids (especially biologics) and suspensions, however, because of the tight clearances between the piston and the cylinder. In piston driven filling machines, the product enters the dispensing cylinder by opening an in-feed valve moving the piston in a reverse direction; closing the in-feed valve; opening a discharge valve; and driving the piston in the opposite direction so that the product is propelled to the nozzle and into the collection container. The volume of the filled product is controlled by adjusting the stroke of the piston. The steps of a piston filling machine are:

- suck back
- rotary valve change position
- nozzle open
- piston forward to discharge solution
- nozzle close
- rotary valve change position

Syringe filling machines are typically valveless rotary piston fillers, although peristaltic and time pressure syringe fillers do exist. Instead of the existence of a solid piston, a portion of the piston body is removed. On the in-feed stroke, the side of the piston with the cavity is rotated to the inlet. The downstroke creates a vacuum, and product enters the pump body. The piston rotates 180 degrees, and the liquid-filled cavity faces the outlet. The pump upstroke occurs, and the product is forced out of the pump. The rotation continues another 180 degrees, and the cycle is repeated.

	Strengths	Weaknesses
Piston pumps with lapped rotary valves	▪ simplicity—three parts; no consumable parts, such as a rubber seal or diaphragm ▪ simplicity of motion ▪ no sophisticated controls required ▪ accurate for small or large fill volumes ▪ reasonably easy to CIP/SIP	▪ may damage shear sensitive products (biologicals) ▪ greater source of metallic particles ▪ push-pull actuation ▪ thorough cleaning required between filling campaigns ▪ must be located in clean environment ▪ high cost of equipment

		▪ handling issues during cleaning—nicking; unable to interchange piston and cylinder ▪ unable to run dry ▪ potential for seizing ▪ leakage varies with input pressure
Piston pumps with rolling diaphragm	▪ clean—few parts in product path ▪ no leakage in product path ▪ gentle to sensitive products since no shear is involved ▪ ability to run sugar based products without seizing ▪ ability to handle slurries ▪ ability to run dry ▪ accurate for small or large fill volumes ▪ pump loads the actuating mechanism, eliminating backlash	▪ special assembly requirements; need highly trained personnel to assemble ▪ one-way valves necessary—duck bill valves ▪ vacuum source required ▪ diaphragm must be discarded and replaced ▪ number of components ▪ pump must be horizontally oriented for CIP/SIP—can affect drainage

[9]

[9] Peterson, A. Filling methods as they apply to parenteral product quality and biopharmaceutical microdosing. In Practical Aseptic Processing: Fill and Finish. Vol 1, Lysfjord, J. ed, Parenteral Drug Association, Bethesda, MD, 2009, 145-165.

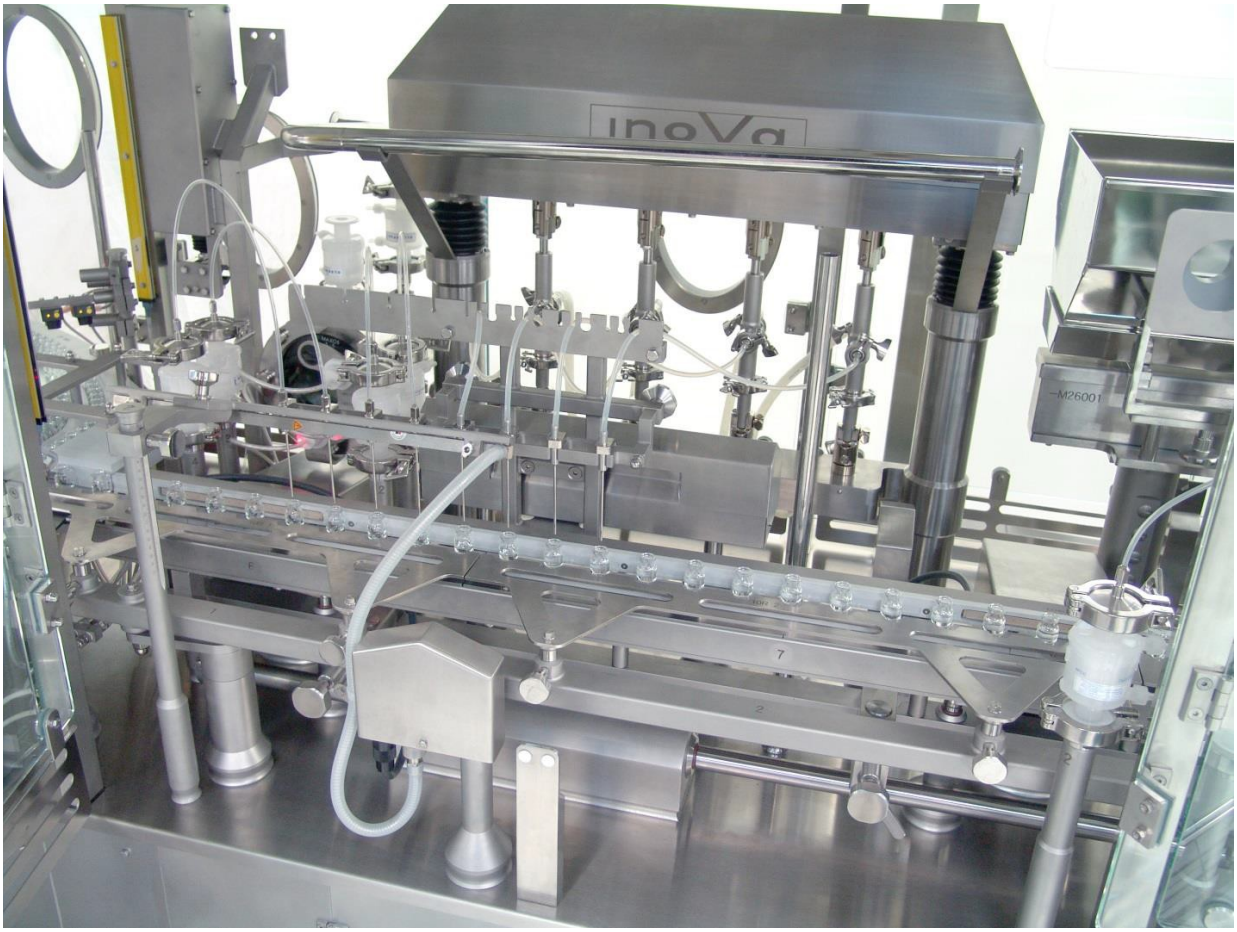


Figure 14-13. Inova vial filling machine with rotary piston pumps and in-line check weighing (courtesy OPTIMA Corporation)

Peristaltic Filling

Peristalsis describes movement of ingested food in the gastrointestinal tract; and this same principle is used for filling machines. Peristaltic filling involves positive displacement, where the solution is contained within a flexible tube fitted inside a circular (rotary) or elongated (linear) pump casing (Figure 14-14). A rotor, with a number of rollers, shoes, or wipers attached to the external circumference, compresses the flexible tube. As the rotor turns or moves, the portion of the tube under compression closes (occludes), thus forcing the fluid to be pumped to move through the tube. Additionally, as the tube opens to its natural state after the passing of the cam (restitution), fluid flow is induced.

Figure 14-14. Inside of a rotary peristaltic pump (courtesy Colanar)

Since there are no moving parts in contact with the fluid, peristaltic pumps are inexpensive to manufacture. The lack of valves, seals, and glands makes them comparatively inexpensive to maintain, and the use of a hose or tube makes for a relatively low-cost maintenance item compared to other pump types. Peristaltic pumps also minimize shear forces experienced by the product solution; this makes them ideal for shear sensitive protein products. They are not as ideal, however, for high viscosity liquids and cannot match rotary piston machines for small volume filling precision.

Typical tubing systems used for filling machines, regardless of mechanism, are silicone rubber, polyvinyl chloride (fallen out of favor due to potential leachables), and fluoropolymer.

	Strengths	Weaknesses
Peristaltic pumps	▪ clean—few parts in product path ▪ easy to maintain and change over ▪ product path easy to CIP/SIP ▪ suitable for completely disposable filling systems ▪ no leakage in the product path ▪ ability to handle sensitive products ▪ ability to handle suspensions and slurries ▪ ability to run dry ▪ easy cleanup for potent products	▪ pulsating flow ▪ accuracy issues due to tubing tolerances ▪ angular position of rotor at start and stop may affect dose ▪ tubing size and shape may change over time ▪ volume may drift over time—can be compensated for with filling machines with automated check weighing capability

[10]

Liquid Filling

There are three main methods for filling liquids into containers with high accuracy: volumetric filling, time pressure dosing, and net weight filling.

Volumetric filling machines employing pistons or peristaltic pumps are most commonly used (e.g., the Colanar filling machine [Figure 14-15]). This filler is best suited for small batch filling of 2 mL (13 mm openings) to 100 mL vials/bottles with 20 mm openings. Filling speeds for 2 mL to 20 mL vials are dependent upon both the size of the vial and the vial handling equipment (filling speed for this type of equipment may range between 40 and 120 vials per minute depending on setup).

[10] Peterson, A. Filling methods as they apply to parenteral product quality and biopharmaceutical microdosing. In Practical Aseptic Processing: Fill and Finish. Vol 1, Lysfjord, J. ed, Parenteral Drug Association, Bethesda, MD, 2009, 145-165.

Figure 14-15. Syringe filling system
(courtesy Colanar)

Stainless steel syringes are required with viscous liquids, as glass syringes are not strong enough to withstand the high pressures developed during delivery.

When high-speed filling rates (currently up to 800 per minute for vials and 60,000 per minute for 0.5 mL to 1.0 mL long syringes) are desired but accuracy and precision must be maintained, multiple filling units can be joined together and electronically coordinated. When the product is sensitive to metals, a peristaltic pump filler may be used since the product only comes into contact with silicone rubber tubing. While there might be some sacrifice of filling accuracy (± 3 percent with < 0.5 mL fill volumes), technology now uses 100 percent check weighing of filled containers, so filling accuracy is still quite good.

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

The Inova VFVM2428 model (Figure 14-16) is an example of a time pressure filling machine designed for fast filling of large batches. It can fill vial sizes from 2 mL to 100 mL at filing rates of 60 to 300 per minute.

Figure 14-16. Inova vial filling machine with time pressure pumps and in-line check weighing (courtesy OPTIMA Corporation)

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

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. This is a manual process in which the operator is in close proximity to the container being filled, so good aseptic technique is necessary to maintain product sterility. Clean, sterile, and disposable assemblies operating on the same principle have particular usefulness in hospital pharmacy or experimental operations (Figure 14-17).

The United States Pharmacopeia (USP) requires that each container be filled with a sufficient volume in excess of the labeled volume so as to ensure withdrawal of the labeled volume. The USP also provides a table of suggested fill volumes (sec. 1151: Pharmaceutical Dosage Forms).

Figure 14-17. Tabletop vial filling system with disposable wetted parts (courtesy Colanar)

Pre-filled Syringe Processing and Filling

Syringes are cleaned, sterilized (by ethylene oxide or radiation), and sealed by the manufacturer with a puncture-proof lid before they are delivered to the finished product manufacturer. Syringes are contained in a plastic, double wrapped tub system in order to maintain sterility. The transfer of these tubs and sterile syringes from a receiving area into the aseptic filling area presents a challenge with respect to maintaining sterility. Typically, the outer bag wrap is removed within a Grade C/ISO 8 area, and the inner bag wrap is sanitized (alcohol or hydrogen peroxide vapor) before moving into the aseptic area. Low energy e-beam radiation is now an alternative for the surface decontamination process and increases the level of sterility assurance in the transfer of pre-sterilized syringe tubs into the aseptic area. In the aseptic area, an operator removes the lid of the tub and places it on the filling line. Syringes are filled row by row with precise filling volumes (can be accurate within 0.1 mL); the rubber plunger is then accurately inserted at the pre-determined location within the syringe barrel to assure accurate delivery volume (Figure 14-18). Syringe fillers are designed to first fill the sterile syringes then insert the sterile stopper. If the stopper insertion rods or tubes are not properly aligned, the product could potentially contact the rods and tubes and break the glass. Syringe fillers can typically fill 0.5 to 20 mL syringes at rates between 60 and 600 syringes per minute.

Figure 14-18. Inova syringe filling machine needle and plunger assemblies (courtesy OPTIMA Corporation)

Cartridge Filling

Bausch + Stroebel cartridge filling machines are capable of filling up to 3 mL cartridges at rates of 300 per minute. With cartridges, the rubber plunger is first inserted into a predetermined location within the barrel of the siliconized cartridge. The product is inserted, typically with either a two- or three-shot fill so there is no significant head space, and the cartridge is sealed

with a sterile, rubber septum within an aluminum cap. Excessive air space in a cartridge affects dose accuracy when the contents of the cartridge are ejected through a pen delivery system.

Solid Filling

Sterile solids, such as antibiotics, are more difficult than liquids to subdivide evenly into containers. The rate of flow of solid material is slow and often irregular. Even though a container with a larger diameter opening is used to facilitate filling, it is difficult to introduce the solid particles, and the risk of spillage is ever-present. Furthermore, 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.

Dose uniformity and accuracy are also concerns with powder filling. These are functions of both the engineering of the powder filling machine and the particle size characteristics dictated by the methods employed during the production of the solid. Other challenges with powder filling include the control of relative humidity during filling and the minimization of foreign particle contamination.

Some sterile solids are subdivided into containers by individual weighing—a slow process. A scoop is usually provided to aid in approximating the quantity required; however, the quantity in the container is ultimately weighed on a balance. When the solid is obtainable in a granular form so that it flows more freely, other methods of filling may be employed. In general, these involve the measurement and delivery of a volume of the granular material that has been calibrated in terms of the weight desired. An adjustable cavity in the rim of a wheel is filled by vacuum, with the contents held by vacuum until the cavity is inverted over the container. The solid material is then discharged into the container by a burst of sterile air.

The Perry Accofil® system was developed as a solution to the problem encountered by the pharmaceutical industry with the introduction of penicillin several years ago. Until the availability of such a machine, powders were manually weighed; and as a consequence, problems arose with both sterility assurance and operator exposure to powder particles. With the Perry Accofil® system, a metering cylinder contains an adjustable piston with a porous filter head that is impervious to powder but passes air. The piston head forms the bottom of the cylinder and can be adjusted to provide a desired powder volume. The vacuum is applied through the piston/filter, which causes the powder to be drawn into the cylinder from a bulk supply hopper. Since the filter material of the piston head passes air but not powder, a compact slug of powder material is formed in the cylinder by the vacuum. When the cylinder is withdrawn from the bulk hopper, a mushroom of powder rises with the filled cylinder. The excess powder is doctored off the end of the cylinder and remains in the hopper. Here it is broken down into its original powder form since the vacuum is no longer applied to it. The powder slug formed is then discharged by replacing the vacuum behind the porous filter with a pulse of low pressure air. Perry claims a fill accuracy of +/- 0.5 percent to 2.0 percent.

Filling of sterile powders invariably offer more challenges than filling of liquids. The issues or problems that may occur in the filling of solids include:

- dose accuracy from container to container
- content uniformity of the solid has more than one component

- environmental humidity not controlled
- maintaining aseptic conditions, especially with particulate controls
- increased probability of particulate matter in the product

Suspensions/Other Dispersed System Filling

The main issues or potential problems that may occur with dispersed systems include:

- maintaining dose homogeneity from container to container
- validation of dose homogeneity, especially with higher product viscosities
- clogging of filling needles/nozzles
- batch size
- aseptic formulation
- particle size reduction under aseptic conditions

Maintaining dose homogeneity during filling operations is an immense challenge. Dose homogeneity is dependent upon the ability of the recirculation system, which supports the filling system, to prevent suspension particle settling or emulsion globule interaction and growth. The primary way of maintaining dose homogeneity is filling of the recirculated suspension. Some form of in-process check is performed to assure suspension homogeneity during the filling process.

Check weighing

All filling operations must be checked for accurate dose filling both prior to the start of th filling operation (to make proper initial adjustments) and during filling (by checking fill volumes periodically to ensure that predetermined volumes or weights are within specifications).

There are several check weighing methods (focus on vials)[11]

- manual check weighing
- robotic check weighing of a single container
- robotic check weighing of a full container set
- 100 percent non-contact check weighing

Regardless of the check weighing method used, control charts are established and monitored during a filling operation. Each filling operation has a target fill volume or weight with upper and lower acceptance limits. Typical fill requirements are $\pm$ 0.5 percent of the target fill volume for each and every filling nozzle; however, the smaller the fill volume the more difficult it is to maintain these tight tolerances. For example, a target fill weight might be 5.0 grams, with the upper limit being 5.1 grams and the lower limit being 4.9 grams. A fill volume of 0.2 grams would require a fill tolerance of +/- 0.001 grams. This is difficult to successfully measure and

[11] Peterson, A. Checkweighing fill weight of parenteral product is the heart of process quality, In Practical Aseptic Processing: Fill and Finish, Vol 1, Lysfjord, J. ed, Parenteral Drug Association, Bethesda, MD, 2009, 135-144.

practically impossible to maintain, so the tolerances would need to be expanded (e.g., +/- 3 percent). For liquid filled products, the product density (or specific gravity) must be accurately known so that a conversion to weight can be determined. Periodic weight checking is performed, and the data are recorded on a control chart. Filling precision is calculated using the smaller of the following two calculations:

- (upper specification limit−average weight) / 3σ
- (average weight−lower specification) / 3σ

  where 3σ is three standard deviations from the average (mean) weight value where 99.73 percent of all data fall within this range

Advances in vial and syringe filling[12],[13]

While the emphasis of this textbook is a basic approach, certain industrial trends should be addressed.

Flexible Lines

Due to the exceptionally high costs of some new drugs, especially biopharmaceuticals, it is preferable to fill small batches in order to reduce the risk of unacceptable monetary losses in the event of a manufacturing deviation that results in batch rejection. The move toward smaller batch filling has necessitated the requirement for more accurate fills and faster line changeovers. One way that this is accomplished is through the use of single-use, disposable closing systems, in which the entire product path is discarded after use. Another approach is to modify filling designs so that only one change part is required for a vial diameter change. Filling machines are available that have more than one dosing system to increase flexibility for filling a variety of products.

Reduced Customization

A number of companies have experienced numerous problems with highly customized filling equipment; thus, standardization of filling machines has seen a resurgence. Standardization includes vendor selection, programmable logic controls (PLCs), human machine interfaces (HMI, touch screens), component transfer systems, filling method, and design of rapid access barrier systems (RABS) or isolator enclosures. Reduced customization has resulted in faster line fabrication, shorter factory acceptance testing (FAT), and reduced risk associated with startup, site acceptance testing (SAT), installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ). Also, maintenance is simpler and there is reduced need for spare parts.

Integrated and Compact Lines

The pharmaceutical industry is moving toward single-sourced, integrated filling lines. An example would be the BOC Edwards production freeze-dryers and associated automatic loading

[12] Heyman, P., Recent trends in vial and syringe filling, Pharm. Processing, July, 2009, 18-23.
[13] Auerbach, M., Aseptic technologies process revolutionizes closed vial filling, Pharm Processing, May, 2009, 8-11.

and unloading systems. For low to intermediate production volumes, compact lines, such as IMA's Modular Aseptic Compact System, have been implemented and include vial washer, depyrogenation oven, and filling machine all integrated as one complete unit.

Filling machines for integration with barrier isolators or rapid access barrier systems

Integrations between filling machines and barrier isolators are the expected norm in pharmaceutical manufacturing, where streamlined filling machines have been produced to fit precisely into these isolator systems to optimize airflow, aid in sterilant distribution, be ergonomic with the glove ports, facilitate removal of waste, and make it simpler to remove the source of jams. Such filling machines are linear fillers with small widths. Vial transport systems to these isolator filling machines have been improved to allow complete exposure to sterilizing gases, typically vapor phase hydrogen peroxide. Electron beam tunnels are available to surface sterilize tubs of prefilled syringes directly feeding a syringe filler. Both upstream automated bag opening and downstream automated tub lid removal have been integrated to provide greater separation of operators from the process.

Higher grade vial capping

Because of European Union requirements for Grade A air supply over capping operations, capping machines are available with restricted access barrier system (RABS) enclosures that target unidirectional downward airflow over the capping head, sorting bowl, and chute.

Integration of external vial washing

Vial washing machines can be purchased to wash the vial exterior after filling in order to remove potent compounds on the exterior surface for added operator and user safety. Such machines aim water rinses so that the vial caps are not wetted, and filtered compressed air is used to dry the vials. External vial washing can also aid in the removal of cosmetic defects.

Closed vial filling systems

Aseptic Technologies developed the Crystal® Closed Vial Filling System (CVFS), where a ready-to-fill plastic (cyclo-olefin copolymer) vial and thermoplastic elastomer are molded in a Grade A clean room, assembled robotically, then gamma-irradiated prior to delivery to the manufacturer. The specialized filling machine needle pierces the stopper, liquid is filled into the vial, the needle is withdrawn, and the piercing trace is laser resealed to restore closure integrity. A cap designed to keep the stopper surface protected until use is placed by snap-fit. All of these operations are conducted inside a Closed Vial Filling System that ensures Grade A environmental control.

Table 14-4. Advantages and disadvantages of sterile product filling methods

Filling Mechanism	Advantages	Disadvantages
Time pressure	▪ clean—few parts in the product path ▪ easy to maintain and change over ▪ product path easy to CIP/SIP ▪ no leakage in product path ▪ ability to handle sensitive products ▪ ability to run dry ▪ accurate for small or large fill volumes ▪ possible self-adjustment of fill volumes between fill cycles at line speed	▪ pressure change sensitivity ▪ pressure control and monitoring required ▪ temperature sensitivity ▪ viscosity sensitivity ▪ expensive parts for changeover of fill volume
Fill-by-weight	▪ clean ▪ easy to maintain and change over ▪ easy to clean and to CIP/SIP ▪ no leakage in product path ▪ ability to handle sensitive products ▪ ability to run dry ▪ real time fill volume control ▪ 100 percent documentation of fill volumes ▪ accurate for small and large fill volumes	▪ high cost of scales and control system ▪ maintenance (potential for spillage on scales) ▪ complex container handling ▪ longer fill times ▪ accuracy for small fills decreases with fill volume reduction ▪ dripping or spills can damage scales—protection of scales complicates the system
Piston pumps with lapped rotary valves	▪ simplicity—three parts; no consumable parts, such as a rubber seal or diaphragm ▪ simplicity of motion ▪ no sophisticated controls required ▪ accurate for small or large fill volumes ▪ reasonably easy to CIP/SIP	▪ may damage shear sensitive products ▪ greater source of metallic particles ▪ push-pull actuation—actuating mechanism must be backlash free ▪ thorough cleaning required between filling campaigns ▪ must be located in clean environment ▪ costs ▪ handling issues during cleaning—nicking; cannot interchange piston and cylinder ▪ unable to run dry ▪ potential for seizing

		▪ leakage varies with input pressure
Piston pumps with rolling diaphragm	▪ clean—few parts in product path ▪ no leakage in product path ▪ gentle to sensitive products since no shear is involved ▪ ability to run sugar based products without seizing ▪ ability to handle slurries ▪ ability to run dry ▪ accurate for small or large fill volumes ▪ pump loads the actuating mechanism, eliminating backlash	▪ special assembly requirements; need highly trained personnel to assemble ▪ vacuum source required ▪ diaphragm must be discarded and replaced ▪ number of components ▪ pump must be horizontally oriented for CIP/SIP—can affect drainage
Peristaltic	▪ clean—few parts in product path ▪ easy to maintain and change over ▪ product path easy to CIP/SIP ▪ no leakage in the product path ▪ ability to handle sensitive products ▪ ability to handle suspensions and slurries ▪ ability to run dry ▪ easy cleanup for potent products—best of all filling systems	▪ pulsating flow ▪ accuracy issues due to tubing tolerances; angular position of rotor at start and stop; change of tubing size/shape over time; and check weigh and adjustment must compensate for volume drift

Stoppering

Ampoules do not require rubber closures and are sealed with a flame. Vials are closed with rubber stoppers (for vials containing solution to be freeze-dried, the stopper is partially inserted into the vial opening), and syringes and cartridges are closed with rubber plungers at the distal end (with rubber septa sealing the proximal end except for staked-needle syringes). Rubber stoppers and plungers need to be lubricated with either applied silicone oil/emulsion or with special coatings (fluoropolymer coating placed on the closure by the manufacturer) that permit rubber units to move easily from the hopper along stainless steel tracks or rails to the openings of the primary containers (Figure 14-19). Filling efficiencies are often more dependent on the stoppering process than on the actual filling process, as there are tendencies for rubber closures to slip off or pop off the openings of vials. For syringes and cartridges, the placement of the rubber plunger is dictated by the desired position of the plunger within the barrel of the syringe or cartridge used to deliver the claimed volume of product.

Problems encountered during stoppering include:

- either too little or too much silicone on stoppers
- misaligned or bent syringe stopper insertion rods or tubes
- stoppers become jammed on the track

- improper head space (syringes)
- stoppers not completely seated

Figure 14-19. Vial filling system depicting stoppering process

(courtesy Colanar)

Sealing/Capping

Ampoule containers should be sealed as soon as possible to prevent the contents from being contaminated by the environment; this is accomplished by melting a portion of the glass neck.

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

Pull-seals are created by heating the neck of the ampoule below the tip, leaving enough of the tip for grasping with forceps or other mechanical devices. The ampoule is rotated in the flame from a single burner. When the glass has softened, the tip is grasped firmly and pulled quickly away from the body of the ampoule, which continues to rotate. The small capillary tube thus formed is twisted closed. Pull-sealing is slower, but the seals are surer than those created by tip-sealing. Powder ampoules or other types having a wide opening must be sealed by pull-sealing. Fracture of the neck of ampoules during sealing may occur if wetting of the necks occurred at the time of filling. Also, wet necks increase the frequency of bubble formation and unsightly carbon deposits if the product is organic.

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

Vials and bottles are closed by inserting a rubber closure (stopper) into the opening. This must be accomplished as rapidly as possible after filling and with reasoned care to prevent contamination of the contents. The large opening of vials and bottles makes the introduction of contamination much more probable than with ampoules. Therefore, during the critical exposure time, the open containers should be protected from the ingress of contamination, preferably with a blanket of HEPA-filtered laminar airflow. The closure must fit the mouth of the container snugly enough so that its elasticity seals rigidly to slight irregularities in the lip and neck of the container. However, it must not fit so snugly that it is difficult to introduce into the neck of the container. Preferably closures are inserted mechanically using an automated process, especially with high-speed processing. When the closure is positioned at the insertion site, it is pushed mechanically into the container opening. To reduce friction and enable the closure to slide easily through a chute and into the container opening, the closure surfaces are either halogenated or treated with silicone.

When small lots are encountered, manual stoppering with forceps may be used, but such a process poses greater risk of introducing contamination than automated processes. This process can serve as a valuable test for the evaluation of operator aseptic techniques but is not recommended for product filling and stoppering.

Rubber closures are held in place by means of aluminum caps (seal). The caps cover the closure and are crimped under the lip of the vial or bottle to hold them in place. The caps are designed so that either the outer layer of double-layered caps or the center of single-layered caps can be removed to expose the center of the rubber closure without disturbing the band that holds the closure in the container. Rubber closures for use with intravenous administration sets often have a permanent hole through the closure. In such cases a thin rubber disk overlaid with a solid aluminum disk is placed between an inner and outer aluminum cap, thereby sealing the hole through the closure. The closure cannot be removed without destroying the aluminum cap; this makes the container tamper-proof. Therefore, an intact aluminum cap is proof that the closure has not been removed either intentionally or unintentionally. Such confirmation is necessary to ensure the sterility and overall quality of the contents. Container-closure integrity

testing has become a major focus for the industry due to emphasis by regulatory agencies. Container-closure integrity measures the ability of the seal between the glass or plastic container opening and the rubber closure to fit, remain tight, and resist any ingress of microbial contamination during product shelf life. A relatively recent trend, although now standard practice, is the requirement that sealing of vials and other containers be accomplished under Class 100/Grade A/ISO 5 clean room air (the air meets Grade A requirements only under static conditions). Formerly such sealing occurred in unclassified environments.

Lyophilization

Materials can be dried using high temperatures to drive off solvents; however, high temperatures can increase chemical decomposition, and products can be damaged. Freeze-drying, on the other hand, takes place at sub-zero temperatures, and chemical decomposition is minimized. Another benefit of freeze-drying versus high temperature drying is the fact that a freeze-dried product has a much higher surface area, so when solvent is added back to the product, a relatively quick reconstitution can generally be achieved.

A typical lyophilization cycle consists of several distinct phases, each designed for a different purpose. The different stages of freeze-drying are listed below and discussed in more detail.

- freezing
- annealing (not always performed)
- primary drying
- secondary drying

Figure 14-20 depicts a typical freeze-dried vial of an injectable pharmaceutical product.

Figure 14-20. Lyophilized vial of injectable pharmaceutical product

Basic theory

It is understood both how water behaves as a function of temperature and the state it is in at that temperature. Below 0°C water exists as a solid (ice); above 100°C water exists as a gas (steam); and between these two temperatures water exists as a liquid. What is not so commonly known is that changes in the physical state of water (solid, liquid, and gas) can also be achieved as a function of changing pressure. Pressure and temperature effects on water can be better understood by viewing a phase diagram of water as illustrated in Figure 14-21.

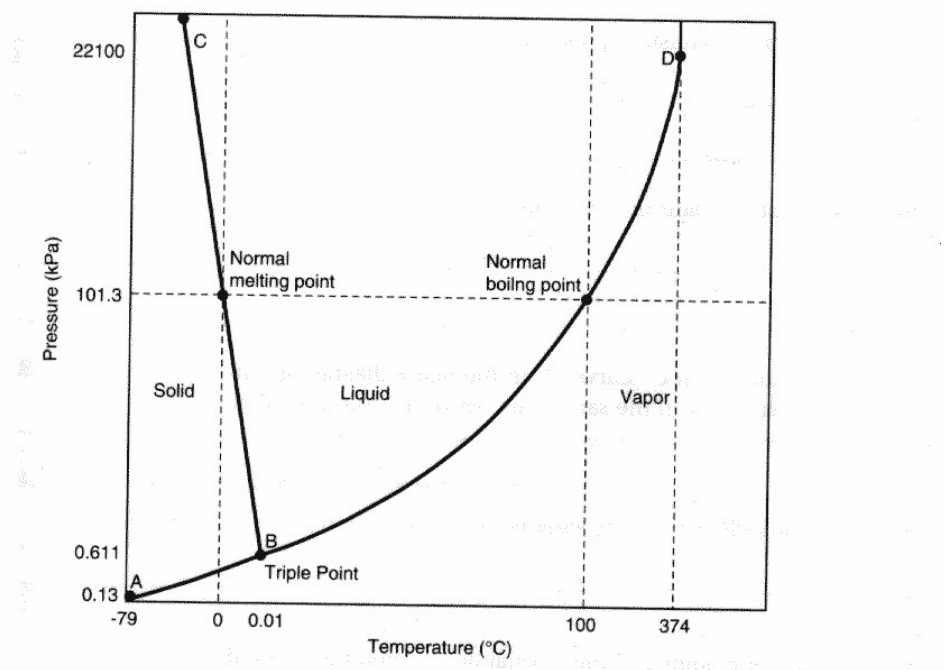


Figure 14-21. Phase diagram of water

As depicted in Figure 14-21, there are numerous variations of temperature and pressure than can be used to keep water in a fixed state. For example, by raising the pressure over water, it is possible to keep water in the liquid state at temperatures well above 100°C. Note that the solid lines in the graph represent that the adjacent phases are in equilibrium with each other at that temperature and pressure combination and there are many combinations where this exists. The only fixed point on this phase diagram is at point B on the graph, referred to as the Triple Point. There is only one combination of temperature and pressure where all three forms of water (liquid, ice, and steam) exist in equilibrium with each other. The triple point is at a temperature of 0.01°C and a of pressure 0.611 kPa.

Figure 14-22 is the same phase diagram as Figure 14-21; however, it has been highlighted to demonstrate what is occurring during the primary drying phase of freeze-drying. The blue star on Figure 14-22 represents the initial solution at room temperature (25°C) and atmospheric pressure (101.3 kPa). In the first step of freeze-drying, the sample is cooled to a sub-zero temperature to freeze. Note that the sample crosses the liquid-solid line and forms a solid

(indicated by arrow 1). Once the sample is frozen, a vacuum is created, and the pressure is reduced (indicated by arrow 2). Once the vacuum set point has been achieved, the sample is warmed (indicated by arrow 3). Notice that as the sample is warmed it crosses the solid-to-vapor phase line in the graph. As it does this, the ice is going through sublimation, where it is converted from a solid directly to a gas while skipping the liquid phase. The vapor is removed from the chamber, and the sample is freeze-dried.

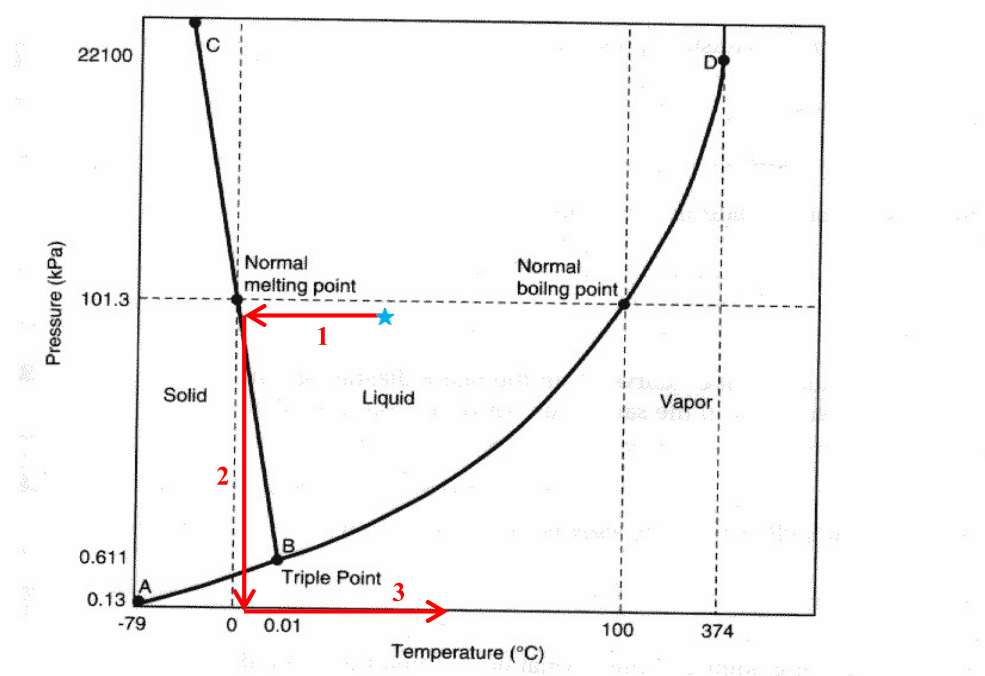


Figure 14-22. Phase diagram of water when freeze-drying

Brief history

Freeze-drying was unknowingly being practiced many years ago by indigenous people living in the colder environments throughout the world. A large part of the diet of many of these groups of people was fish, and to sustain them through the harshest parts of winter they had to devise a method with which to preserve a portion of their catch. Fish were caught and frozen by leaving them outside. They were then hung on racks and slightly warmed by the sun; and since the humidity was extremely low in such a cold environment, the ice in the frozen fish slowly sublimed, and the fish were freeze-dried. This was a slow process and took weeks to complete due to the fact that these samples were drying through what is known as diffusive flow transport of water vapor. In modern freeze-drying, the equipment used allows freeze-drying to be performed using bulk flow of water vapor, which is a much more efficient and faster process.

Many people are practicing freeze-drying in their homes without knowing it. For example, if a poorly wrapped piece of meat is purchased, placed in the freezer, and forgotten for several months, the result is essentially a shrunken piece of freeze-dried meat that is similar to a piece

of shoe leather. The humidity in the freezer is extremely low, so by the principles of equilibrium, the ice in the meat sublimes over time by diffusion to freeze-dry the meat.

During the 19th century there was an upsurge in the study of microorganisms by biologists; however, they experienced difficulties studying these over long periods of time. The means of increasing long-term stability at the time was drying using heat; and as many of these molecules were heat-labile (damaged by heat), this type of preservation was not an option. It was near the same period, however, that scientists and engineers discovered how to liquefy atmospheric gases, which led to refrigeration. In 1890, Altman reported that he was able to preserve tissue at -20°C by slowly drying at atmospheric pressure. And in 1905, two scientists first reported that they were able to freeze-dry a sample of animal tissue under a slight vacuum by using a chemical pump—the vacuum created in a closed container through a reaction of several different chemicals.

Freeze-drying did not become commercially practical until the occurrence of two major historical events—the invention of a mechanical vacuum pump to achieve high vacuum levels and the need for stable human plasma after World War I.

During World War I, soldiers with non-life threatening wounds were stabilized by the intravenous administration of plasma to counteract blood loss. After stabilization in the field using plasma, the wounded soldiers were transported back to a field hospital for treatment of their wounds. One of the issues encountered with this procedure, however, was that supplies of plasma were extremely limited due to the short shelf life associated with it. Many of soldiers bled to death from a non-life threatening injury due simply to the lack of available plasma. After the war, in an effort to avoid similar issues in the future, the United States government placed an emphasis on producing stable plasma. Thereafter the commercial manufacture of freeze-dried plasma began; and this, in turn, changed the course of events during World War II, as more lives were saved.

The equipment and systems for freeze-dryers have improved over the years; however, the key components comprising the earliest systems are still in place.

Equipment and systems

Most modern freeze-dryers utilize the following systems (except manifold dryers) and have utilized these same systems since the beginning of commercial freeze-drying in the early 1900s.

Condenser

The condenser is an extremely cold coil or plate where water vapor created during sublimation re-condenses and collects away from the product. Figure 14-23 is an example of a condenser chamber containing condenser plates.

Figure 14-23. Condenser chamber with condenser plates (courtesy SP Scientific)

Temperature Controlled Shelves

The shelves in a freeze-dryer have hollow channels inside them through which thermal fluid is pumped. The thermal fluid is heated and cooled using a heat exchanger.

Cooling Systems

The low temperatures required for the condenser and shelves are provided by either one of two separate cooling systems. Traditional systems are comprised of a standard compressor-based refrigeration system (several types exist). An alternative to the compressor-based cooling system is the use of a liquid nitrogen system. This is a simple system with no moving parts other than a small pump to move the liquid nitrogen through the system. This type of cooling system requires little maintenance, although a constant supplyof liquid nitrogen must be available.

Vacuum Systems

An extremely high vacuum is required for freeze-drying, so special vacuum pumps are required. Rotary vane pumps have a roughing pump (low vacuum stage) and a high vacuum stage. Figure 14-24 depicts dual rotary vane pumps attached to a freeze-dryer.

Figure 14-24. Dual rotary vane pumps (courtesy SP Scientific)

Controlling Systems

All modern freeze-dryers are operated using computer-based control systems. Software programs allow the precise run to be entered and monitored. Alarms can be programmed to notify the operator if something is not functioning properly where the cycle and/or equipment are concerned. Figure 14-25 depicts the synoptic screen displaying the different systems being monitored by the computer.

Cleaning and Sterilizing Systems

Depending upon the types of products being freeze-dried, some dryers incorporate a cleaning system that allows for easy cleaning of the dryer. Strategically placed spray balls and spray nozzles direct heated water to various locations to rinse off contaminants and/or residual product. Freeze-dryers used to dry injectable drug products or tissues for implantation require a clean in place (CIP) system, incorporating water for injection (WFI) and steam in place (SIP) to sterilize. Dryers can also be sterilized using vaporized hydrogen peroxide (VHP) or ethylene oxide, although these types of systems require special handling and containment of hazardous gases.

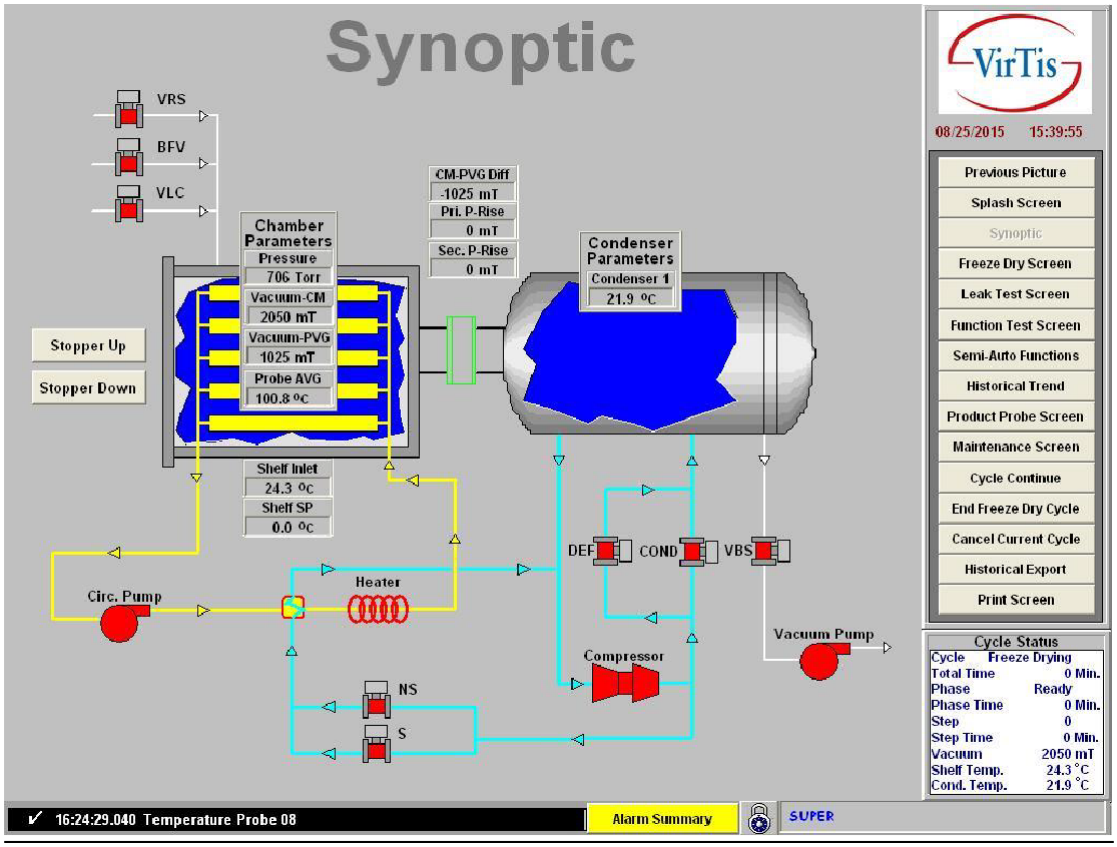


Figure 14-25. Synoptic screenshot

Types of commercial freeze-dryers

Manifold Freeze-dryers

This is the simplest and most cost-effective type of freeze-dryer. Samples for use with this dryer are pre-frozen in a separate freezer, and attached to the manifolds where a vacuum is applied. The system stays frozen, as sublimation is endothermic and cools the sample, and the warmth to the vials is supplied by the outside air. Precise control of sample temperature cannot be achieved with this type of freeze-dryer. A manifold freeze-dryer is depicted in Figure 14-26.

Figure 14-26. Manifold freeze-dryer (courtesy SP Scientific)

Development Scale Freeze-dryers

This type of dryer is used in the development lab to develop lyophilization cycles that are then scaled up to larger dryers for manufacturing. This type of dryer typically has additional sensors onboard, such as a Pirani gauge, that allow the development scientist to better develop a fully optimized cycle for the product. Many of these dryers have a sample thief, which is a separate chamber attached to the main door of the sample chamber. There is trap door between these two chambers, and when a vacuum is applied to the small chamber and reaches equilibrium with the sample chamber, the trap door can be opened and samples can be removed for testing without stopping the run. A development scale freeze-dryer with a sample thief is depicted in Figure 14-27.

Figure 14-27. Development scale freeze-dryer (courtesy SP Scientific)

Pilot-scale Freeze-dryers

These are smaller versions of the large, commercial-scale freeze-dryers. These systems have all of the same features as the larger units but are smaller and run fewer samples.

Commercial-scale Freeze-dryers

These are the large, production-scale freeze-dyers that are used to freeze-dry massive quantities of product. The main door of the freeze-dryer is located in the clean room so that vials coming off of the fill line can be loaded into the dryer in a Class A environment. Many of the newer systems have robotic loading systems that aseptically load and unload the vials. These systems can be several stories tall and cost millions of dollars. Figure 14-28 depicts a commercial-scale dryer.

Figure 14-28. Commercial scale freeze-dryer (courtesy SP Scientific)

Tray Dryers

If product is to be dried in bulk rather than in vials, a tray dryer may be used. In these types of systems, bulk solutions, tissues, or food is placed in a tray, which is then loaded onto the shelves of the freeze-dryer. The samples are freeze-dried and then the bulk material is removed from the tray. These types of systems have all of the same components as the commercial-scale freeze-dryer except the shelves do not compress. Many of these dryers are not used for sterile manufacturing, so a sterilizing system is not required.

Attributes of freeze-dried products

Products are freeze-dried to either increase the shelf life or reduce the weight of a product to make it easier and more cost effective to transport. There are several attributes of freeze-dried products that have to be considered by development scientists when developing the formulation and cycle for these products. In the case of injectable drug products in vials, these attributes need to be considered and addressed.

Reconstitution Time

At the time of use, this is the time it takes for the freeze-dried product to dissolve once either WFI or another solvent is added back to the product. Unusually long reconstitution times make it difficult for the physician, nurse, or pharmacist preparing the product, as constant shaking

must occur. A good reconstitution time is two minutes or less to completely dissolve the product.

Extended Stability

Injectable drug products should have at least a two-year shelf life to be considered viable products. Development scientists attempt to achieve this level of stability at room temperature; however, there are some freeze-dried products that must be stored under refrigeration due to their instability. Temperature accelerates degradation, so keeping samples at lower temperatures extends the shelf life.

Appearance and Pharmaceutical Elegance

The appearance of the product after drying, while more important for marketing and sales, does have to be considered by the development scientist. The goal is to achieve a white, solid product (referred to as a cake) with good physical integrity (does not break apart during shipping). This is not always possible, as some drugs are colored, some cakes are shrunken, and some are loose powder on the bottom of the vial.

Residual Moisture

Moisture plays an important role in the stability of freeze-dried products. The higher residual moisture generally speeds up the degradation reactions causing a decrease in the shelf life of the products. For most products a moisture value of 1 percent or less is desirable; however, for some bio-molecules (proteins, monoclonal antibodies, etc.), higher moisture values are required for better stability.

Industries

Industries dealing in products such as food, diagnostic products, and tissues all have additional requirements for their products in regards to attributes, and these requirements must be addressed by development scientists within each industry.

Temperature and vacuum monitoring devices

Accurately monitoring and controlling temperature and pressure during the lyophilization process is absolutely critical in not only effectively drying products but also drying them in a timely manner. Freeze-drying is the most expensive unit operation in a manufacturing process, so operating the freeze-dryer for the shortest possible time is desirable to minimize manufacturing costs. There are several different types of vacuum and temperature monitoring devices that can be used for freeze-drying but not all are created equal in regards to the data obtained and the accuracy and precision of that data. While there are only two different regions where pressure is measured in a freeze-dryer (sample chamber and condenser chamber), there are many areas where temperature is measured and controlled, including product temperature, shelf temperature, and condenser temperature. The following temperature monitoring devices have been used for freeze-drying.

Temperature Sensors

Resistance temperature detectors (RTDs)

RTDs are the older of the temperature monitoring devices but are still in use in some older freeze-dryers. An RTD is composed of a metal wire (typically platinum) that is wrapped around a core (typically ceramic) and covered by an outer layer (typically glass). A current is passed through the wire, and a certain resistance to the current flow through the wire is achieved. As the temperature of the wire changes, the resistance to the current flow changes; this is the basis of operation for the temperature sensor. The system is calibrated as a function of temperature and resistance so that when the wire is exposed to different temperatures, the computer senses the change in resistance and correlates that back to a change in temperature. An RTD is depicted in Figure 14-29. The major advantage of using RTDs for temperature monitoring is that the device gives a linear response in regards to resistance and temperature change. This not only makes the device extremely accurate and precise but also makes calibration easier. The disadvantage of RTDs is that the temperature reported is a function of the temperature across the entire wire. This is not a problem if the sensor is either taped to the surface of the condenser or in the path of the thermal fluid going into or coming out of the shelves; however, this is a substantial disadvantage for probes that are used to measure product temperature where a point sensing device is needed. Another disadvantage to these types of probes stems from their operation—they are self-heating, which results in the samples containing these probes to become non representative of the other vials in the batch.

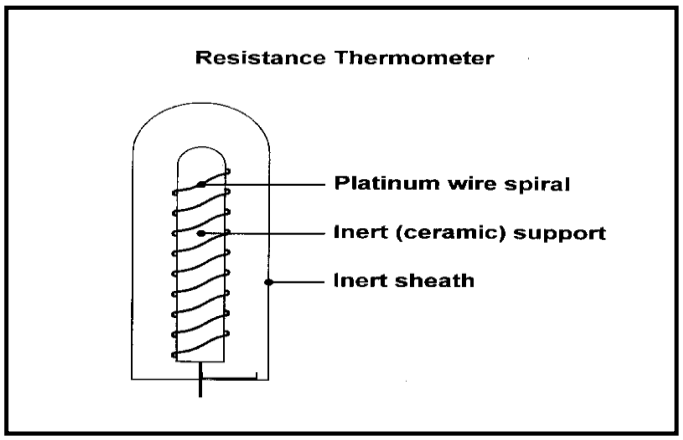


Figure 14-29. RTD temperature probe

Thermistors

Thermistors, like RTDs, are wires that experience a change in resistance as a function of changing temperature. In this case the wires are welded at the tip, so the resistance change is based on the temperature of the tip only, which makes these devices point-sensing in regards to temperature. Unlike RTDs, these wires are semiconductors composed of metal oxides, such as nickel or cobalt oxide. The advantage of thermistors as compared to RTDs is that they are extremely sensitive to temperature change and give a large response to small changes in

temperature. The main disadvantage to thermistors is that their response is exponentially nonlinear, making them extremely difficult to keep within calibration. A thermistor is depicted in Figure 14-30.

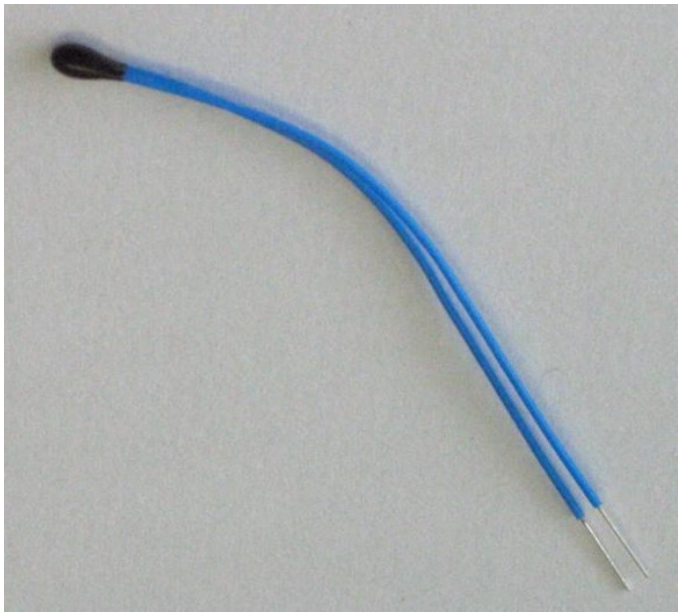


Figure 14-30. Thermistor temperature probe

Thermocouples

Thermocouples are the most widely used temperature monitoring devices in freeze-drying. They are robust, relatively inexpensive, sensitive, accurate, and precise. Thermocouples consist of two different types of wires (different metals) that are TIG (Tungsten Inert Gas) welded at the tip, which produces a small point. Like the thermistor, this device is point sensing, meaning that the temperature reading comes only from the tip of the thermocouple. The most common type used in freeze-drying is referred to as Type T and is composed of one wire made of copper and another made of constantan. As with the other sensors, the temperature reported is due to a change in resistance at the tip of the probe. The temperature response to the change is not as linear as the RTD, but with regular calibration they function accurately. A thermocouple is depicted in Figure 14-31.

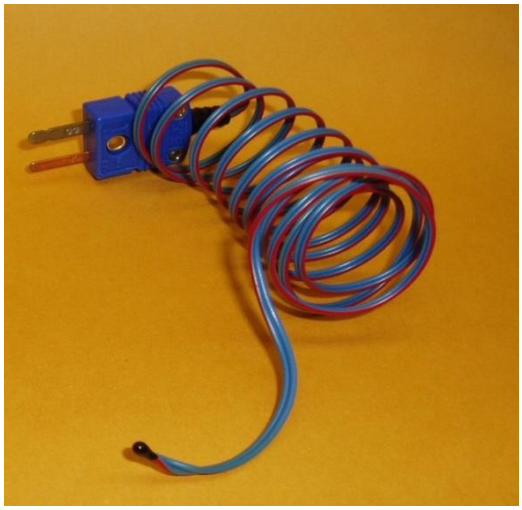


Figure 14-31. Thermocouple probe

Vacuum Sensors

Pirani Gauge

The Pirani gauge is referred to as a hot wire gauge. It is a simple device consisting of a wire (actual set of wires) that is placed in the freeze-drying chamber and exposed to the high vacuum during drying. The wire is heated to a certain temperature and requires a certain amount of energy supplied to it in order to keep it hot. One way the wire loses heat is when the molecules in the surrounding air strike the hot wire and carry away some of its heat. At high vacuum levels most of the molecules in the chamber have been pulled out, so there are far less of them floating around to take heat away from the wire. As such, less energy has to be supplied to keep the wire hot. The amount of the difference in the energy is then correlated back to the pressure changes within the chamber. One disadvantage of these types of gauges are that they are thrown off of set point during primary drying because the water vapor carries a different amount of heat than does the nitrogen gas that was used to calibrate them. Another disadvantage is that they are easily damaged from the stresses of the cleaning and sterilizing required for sterile manufacturing. A Pirani gauge is depicted in Figure 14-32.

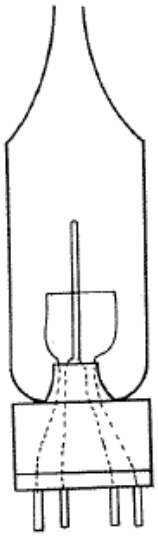


Figure 14-32. Pirani vacuum gauge

Capacitance Manometer

The capacitance manometer senses changes in pressure by using a fluctuating metal diaphragm. This device is a capacitor, and its capacitance is directly proportional to both a geometry factor and the dielectric current of the medium relative to that of air. In the normal operation of a capacitor, the geometry factor is held constant and the dielectric current is varied. For the purpose of freeze-drying, the opposite is true. The thin metal diaphragm is sealed between two fixed electrodes. The reference side of the device is sealed and a small vacuum is created. The other side is open to the lyophilization chamber. As a vacuum is created in the freeze-dryer, the diaphragm stretches (changes geometry) and the capacitance change results in a change in voltage, which is correlated back to pressure on the readout. These types of sensors are not thrown off of set point by water vapor like the Pirani gauges, so they are reading accurate vacuum values during the entire cycle regardless of the type of gas present.

Physical properties of materials

Crystalline vs. Amorphous vs. Mixed Systems

As solutions solidify during freezing, different forms of solid material are produced, and the separation of different phases occurs (Figure 14-33).

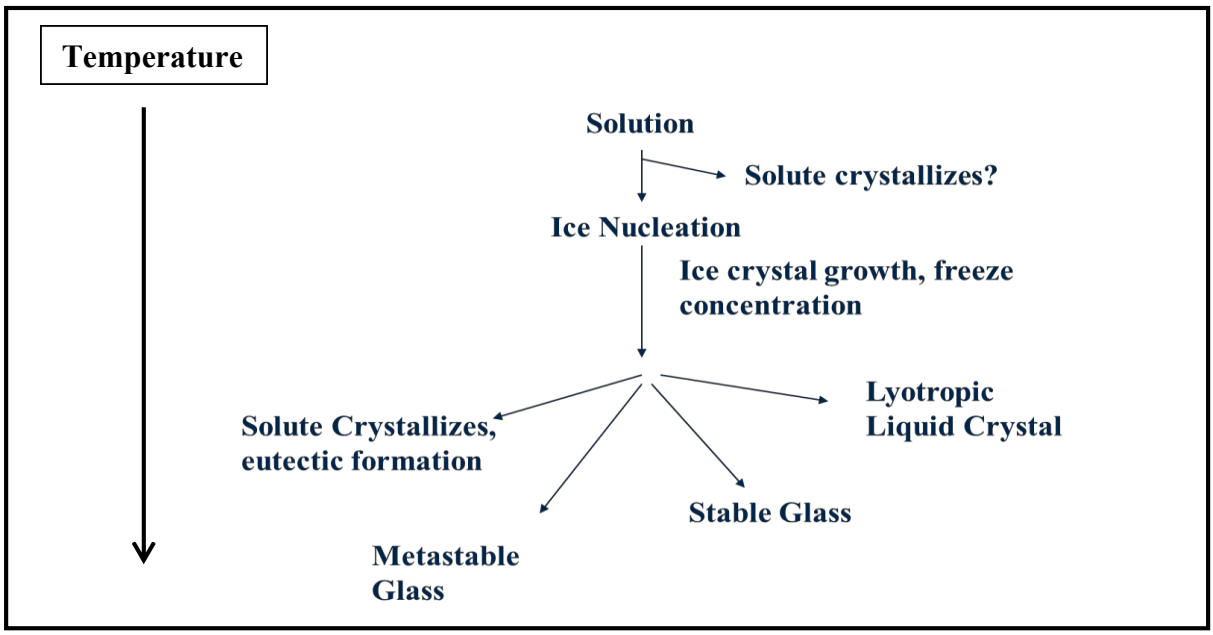


Figure 14-33. Freezing and phase separation

Starting at room temperature at the top of Figure 14-33, the sample is in the solution state. As the temperature decreases to below 0°C, one of two different events occurs. In most cases two water molecules are able to come together, form a stable nucleus, and begin crystal growth. At this time only ice crystals have formed. Everything else that is dissolved in the solution is pushed into what is referred to as the interstitial space surrounding the ice channels. As the ice crystals grow they pull water out of the interstitial space, which causes the interstitial space to super concentrate. Finally, a temperature and concentration are reached where one of four different events occurs. As depicted in Figure 14-38, the concentrated material in the interstitial space forms eutectic, a stable glass, a metastable glass, or a lyotropic liquid crystal. These different phases behave differently during freeze-drying and must be taken into consideration during both formulation and lyophilization cycle development. These are discussed in detail below.

Eutectic

A pure compound melts at a characteristic temperature; however, a eutectic is a solid form of two or more crystalline materials that are in such close contact that they melt like one pure compound. The eutectic in freeze-drying is composed of one or more crystalline compounds combined with ice crystals.

Glass

A glassy or amorphous phase of material is a solid that forms with no order to the molecules. Unlike crystalline species, in which the molecules are organized into a precise structure known as a crystalline lattice, the molecules in an amorphous phase are in complete disorder when they solidify. Glassy phases solidify because they get thicker as they get colder and finally get so thick that they form a solid and can no longer move.

The location of the eutectic or glassy phase and the ice channels is depicted in Figure 14-34.

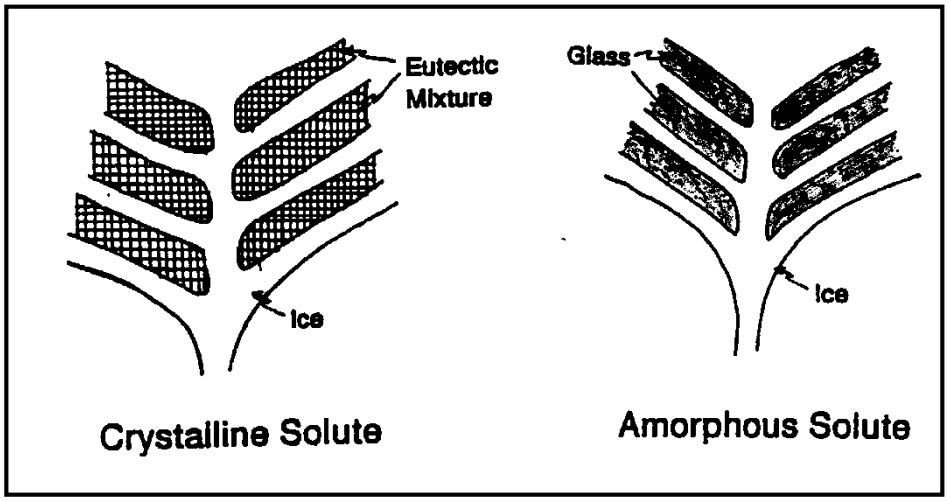


Figure 14-34. Interstitial spaces and ice channels

A clear distinction between the ice in the ice channels and the interstitial space can be observed. It is desirable to have wide ice channels, as these channels provide a conduit to allow the water vapor produced during freeze-drying to escape the product and travel to the condenser where it is collected.

While freeze-dried products can have either eutectics or glasses in their interstitial spaces, it is not uncommon for products to contain both phases in the same product. It should be noted that these two types of phases (eutectic and glassy) freeze-dry differently, so it is imperative for the development scientist to be able to identify which phase is present.

It is also extremely important to determine the temperatures where the eutectic phases and glassy phases melt. So why is it important? As discussed above, there are three basic phases of freeze-drying—freezing (which includes annealing if needed), primary drying, and secondary drying. The main function of primary drying is to remove the pure ice that is located in the ice channels (Figure 14-34). The different phases in the interstitial space (glass, eutectic) have to be rigid enough to support the weight of the freeze-dried solids when the ice is removed. Below the glass transition temperature and eutectic melting temperatures, the interstitial space is a solid. Above these critical temperatures the interstitial space is a fluid and cannot support its own weight when the ice is removed and collapse occurs. An intact freeze-dried solid and a collapsed sample are depicted in Figure 14-35.

Figure 14-35. Collapsed cake and intact cake

Materials characterization techniques

Understanding the type of material formed in the interstitial space (glass, eutectic) and the critical temperature associated with that phase is critical in developing optimal formulations and lyophilization cycles for products.

Principles of Thermal Analysis

As materials pass through the different phases, such as melting, crystallizing, or decomposing, they either absorb heat or release a small amount of heat into the surrounding environment. If a material releases heat, the occurrence is termed an exothermic reaction. If a material absorbs heat, the occurrence is termed an endothermic reaction.

While there are several different instruments that can detect and measure these minute amounts of heat, the most widely used instrument is referred to as a Differential Scanning Calorimeter (DSC). A DSC is depicted in Figure 14-36.

Figure 14-36. DSC
(courtesy/permission TA Instruments)

Rather than functioning by measuring the amount of heat given off or absorbed as materials go through phase changes, the DSC functions by measuring the amount of energy that is required to keep both a sample and a reference material at the same temperature through the phase change. When the sample lid is removed from the instrument, the sample and reference furnaces are exposed (Figure 14-37).

Figure 14-37. DSC sample and reference furnaces
(courtesy/permission TA Instruments)

A small amount of the sample in the liquid state is placed into an aluminum sample pan and the pan is sealed; an empty pan is used for the reference. The pans are loaded into the instrument

and frozen to a low temperature. Afterward, the pans are warmed in order to look for the phase changes in the sample. A typical DSC thermogram is depicted in Figure 14-38.

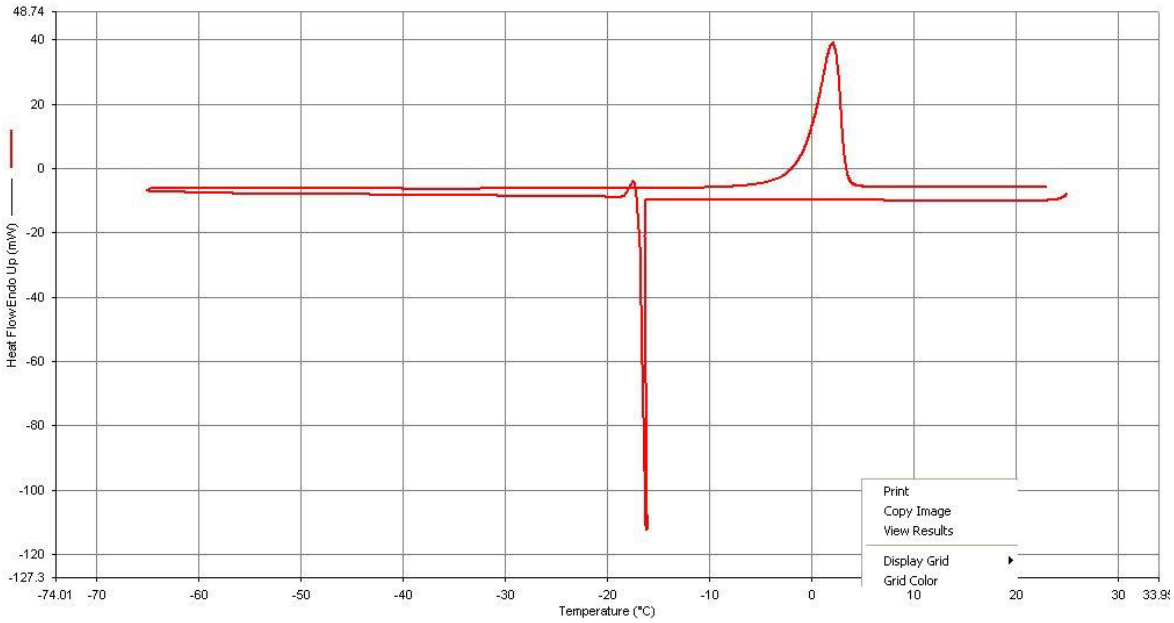


Figure 14-38. Typical DSC thermogram

There are two curves associated with this thermogram—the bottom curve (cooling curve) and the top curve (warming curve). Note that the large peak in the bottom curve is the sample freezing and emitting heat, and the large peak in the top curve is the sample melting and absorbing heat. Only the warming curve is used for the purpose of thermal characterization.

Since the shape of the peak determines what phase is present (eutectic, glass), the critical temperature of where that phase melts can be determined. Figure 14-39 illustrates various peaks from warming curves of the different DSC thermograms.

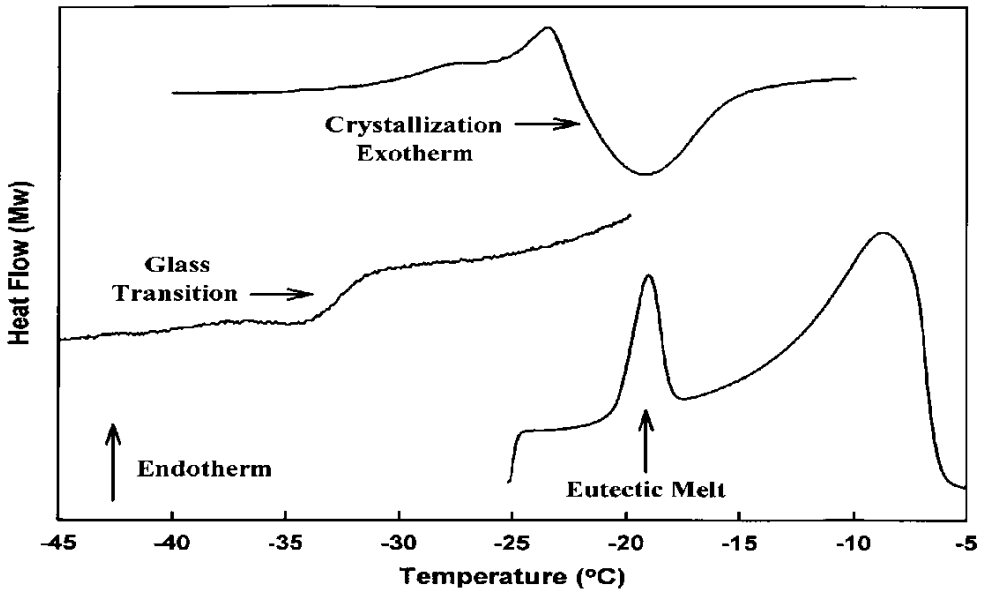


Figure 14-39. Various warming curves

A eutectic melt is observed as a sharp, symmetric peak, while a glass transition manifests itself as a shift in the baseline, which is typically referred to as the S Curve. The upper curve is the result of a metastable glassy phase mentioned previously. The critical temperatures for these phases are determined by the computer software. For the eutectic melt, the computer draws both a tangent to the baseline leading to the peak and a tangent on the leading edge of the peak. The temperature where the tangent line intersects is referred to as the onset of melt temperature and is considered to be the eutectic melting temperature. For the glass transition, the computer draws both a tangent on the leading baseline before the S curve and a tangent to the trailing baseline. The glass transition temperature (Tg) is the midpoint between those lines on the curve.

Another technique routinely used in conjunction with DSC is freeze-dry microscopy. This test supports and supplies additional information about the sample and its critical temperatures. The freeze-dry microscopy system is basically a mini freeze-dryer on a microscope. It allows the technician to freeze-dry a sample in real time and observe it under the microscope. This technique allows the collapse temperature to be determined. The collapse temperature is the temperature where the sample loses structure and cannot support its own weight (Figure 14-34).

The top cover is removed from the stage and a small drop of sample is placed on the stage between two glass coverslips. The cover is returned and the sample is frozen—prior to creating a vacuum and freeze-drying. Images are collected throughout this process. The sample is then warmed to force it into collapse; both the temperature and an image are then recorded. An image collected during collapse is depicted in Figure 14-40.

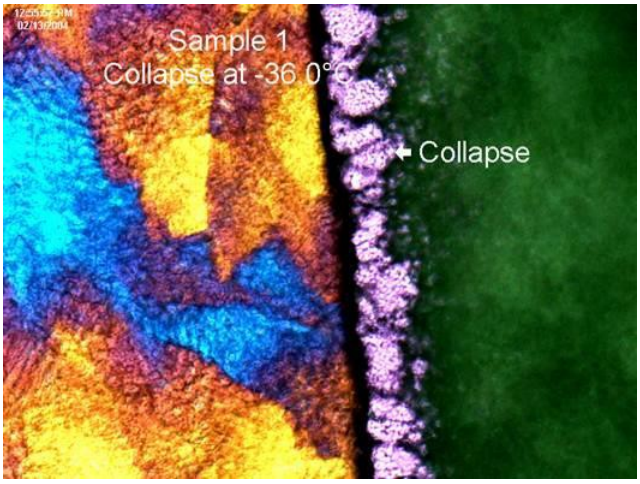


Figure 14-40. Collapse on the freeze-dry microscopy system

These two tests combined have revealed not only a wealth of information about the samples and how they freeze-dry but also pertinent information that will be used to develop an optimized lyophilization cycle.

A freeze-dry microscopy system is depicted in Figure 14-41.

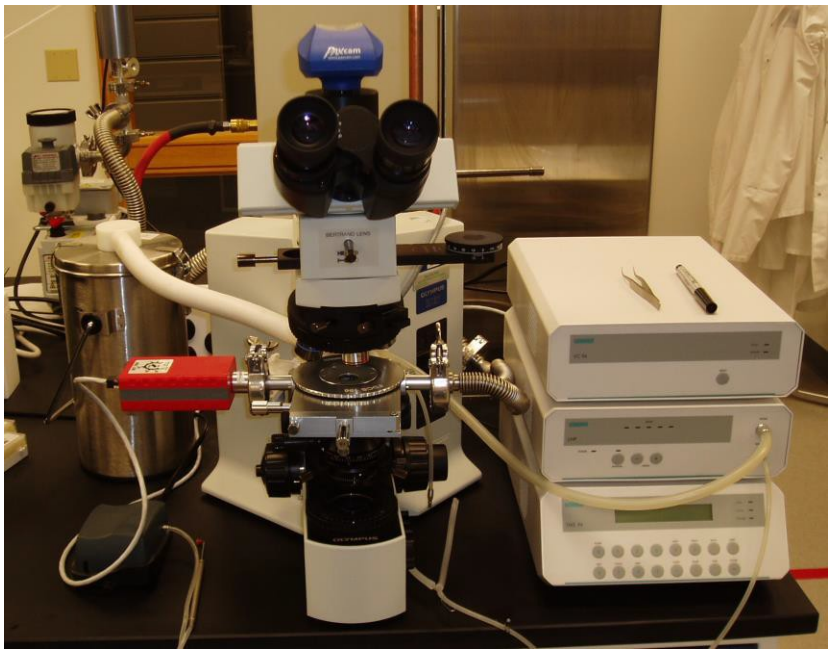


Figure 14-41. Freeze-dry microscopy system (courtesy McCrone Microscopes and Accessories)

The small freeze-dryer can be seen on the microscope stage. The unit has both liquid nitrogen lines connected to cool vacuum lines and an electric heating system to warm the stage. The temperature and vacuum controllers are shown on the right side of the microscope.

Freezing, annealing, and primary and secondary drying

A formulated solution is placed into the freeze-dryer and is frozen on temperature controlled shelves. Prior to samples freezing, which occurs below 0°C due to supercoiling and freezing point depression, water molecules are constantly coming into contact with one another and attempting to form a stable nucleus to start the crystallization process. There is a certain activation energy that must be overcome in order for a stable nucleus to form; and this energy decreases as the temperature continues to decrease. Another method by which to decrease the activation energy and increase the chance of water molecules forming a stable ice nucleus is the addition of either small seed crystals of ice or scratches in vials, both of which provide sharp edges where ice crystals can easily start growing. Once the activation energy has been overcome and conditions become favorable for nucleation, the water molecules form a stable nucleus and ice crystal growth occurs. As mentioned previously, it is only the water molecules that crystalize and form the ice in the ice channels. Everything else (excipients, active ingredients, etc.) is pushed into the interstitial space around the ice crystals where they super concentrate as the water is pulled out to further grow the ice crystals. Finally, a temperature and concentration are reached in the interstitial space where it will solidify forming a glassy phase or crystalline eutectic as described previously. Once the entire product has solidified, primary drying can commence; if an annealing step is necessary it would be initiated at this time.

Annealing the samples accomplishes two things in the frozen product. First, it can change the phase of a sample that has formed a metastable system. Some materials that normally form a stable, crystalline eutectic occasionally fail to crystallize and instead form a glassy phase. This is not the most stable form, and over time the sample will crystallize, so it is important to ensure that this form is in its most stable crystalline form prior to primary drying. Annealing of a frozen phase also causes the ice crystals in the ice channels to grow. This occurs through a process known as Ostwald Ripening and causes some of the smaller ice crystals in the ice channels to melt and form larger ice crystals. This is what is referred to as erasing the thermal history in the samples. Since every sample does not crystallize at the same time and temperature they build up different levels of super-cooling. When the ice crystals eventually form, the formation occurs quickly if a large amount of super-cooling has built up; likewise it occurs slowly if only a small amount of super-cooling has built up. Fast freezing results in extremely small ice crystals, whereas slow freezing results in larger ice crystals.

To anneal the product it is first frozen (ice and interstitial space) and then warmed to a predetermined temperature to allow both the ice to form larger crystals and the interstitial space to crystallize. During annealing, the metastable system in the interstitial space liquefies as it exceeds its glass transition temperature then quickly crystallizes to form the more stable crystalline eutectic phase. The samples are held under these conditions for several hours and then refrozen to solidify completely before starting primary drying. Annealing is technically

considered to be part of the freezing phase of freeze-drying since it is performed at atmospheric pressure prior to creating the vacuum in the chamber.

Once the freezing phase is complete (and annealing if required), the vacuum is started and primary drying commences. The goal of primary drying is to remove the ice in the ice channels, resulting in a dried solid containing numerous small pores (left behind by the ice crystals), which allow water to quickly rush back in when the dried product is reconstituted at the time of use. All substances possess what is referred to as vapor pressure. For example, if a sample of water was placed into a container; a lid was placed on top to seal the container; and a pressure sensor was placed in the container above the liquid, a rise in inside pressure as opposed to the outside pressure would be detected. This is due to what is referred to as the escaping tendency. Some of the water molecules on the surface vaporize, enter the headspace above the liquid, and cause a slight increase in the headspace pressure. If this experiment is repeated with ethyl alcohol instead of water, a much higher vapor pressure would be observed in the headspace in the closed container at the same temperature, as the alcohol has a higher escaping tendency, is more volatile than water, and thus has a higher vapor pressure.

Temperature has a dramatic impact on the vapor pressure of materials. If the aforementioned sealed containers were heated, the pressure would quickly build up to a point at which the containers would explode. Temperature and vapor pressure do not, however, share a direct relationship. As depicted in Figure 14-42, temperature and vapor pressure share an exponential relationship—as the sample temperature is increased, the vapor pressure of the material increases exponentially.

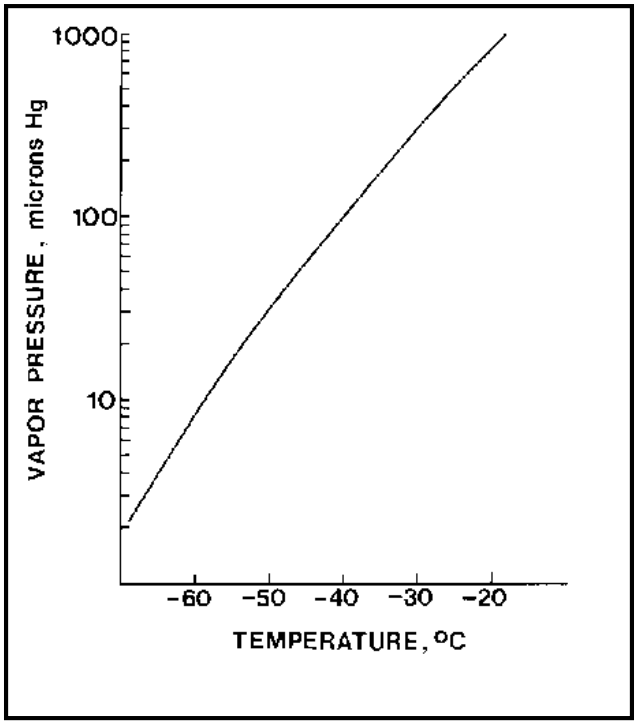


Figure 14-42. Vapor pressure of ice as a function of temperature

Like liquids, ice in the ice channels has a vapor pressure that is affected by temperature as well, and that is the basis of primary drying in freeze-drying. The warmer the frozen sample, the higher the vapor pressure of the ice, the faster the sublimation rate, and the faster it vaporizes and travels to the condenser, resulting in shorter primary drying times.

The driving force in freeze-drying is not temperature but pressure. In order for the water vapor created during primary drying to move from the product to the condenser, there must be a change in pressure—a pressure gradient. The pressure in the product must be higher (created by the water vapor being generated) than the pressure surrounding the condenser where the water vapor collects and refreezes. The vapor pressure of ice as a function of temperature is well understood, so by knowing the temperature of the ice during primary drying, the vapor pressure is known as well. Controlling the vacuum to keep the chamber pressure lower than the vapor pressure of ice ensures that the water vapor consistently flows from the product to the condenser during primary drying. The sample must be kept below its critical temperature; however, during primary drying above this temperature, the interstitial space is a fluid and collapse of the product results when the ice is removed.

Once the ice in the ice channels is removed, the process technically moves into the secondary drying phase. The goal of secondary drying is to remove the residual water in the interstitial space. In secondary drying the sample temperature is increased to enhance the removal of the residual water from the sample. The separate phases in the interstitial space dry differently from one another. If a product is comprised completely of crystalline, eutectic material, it is already 99.99 percent dry at the end of primary drying. This is due primarily to the simple, natural formation of these structures. When materials crystallize they form an extremely precise pattern known as a crystalline lattice. Since there is no room for a water molecule to be incorporated into this lattice (unless a hydrate is formed), the water molecules are pushed outside of the crystal phase. Amorphous glassy phases, on the other hand, do not crystallize and therefore entrap water. The water within these phases is not technically frozen, as no crystalline ice structures have formed. In this phase the water is locked (unfrozen) in an extremely viscous solid (glass). Unlike the crystalline material, which is dry, amorphous phases can contain up to approximately 40 percent of all of the water in the product, all trapped inside an extremely thick, viscous solid. As such, the protocols used to secondary dry these two different materials are drastically different. Conditions can be aggressive for completely crystalline materials, as they are essentially already dry; however, care must be used when dealing with amorphous phases, as the unfrozen water inside the glassy phase must first diffuse to the surface of the glass before vaporizing. This is a slow, time-consuming process, and if it is performed too aggressively, the product can collapse during secondary drying and destroy itself.

Secondary drying is complete when the moisture level reaches a percentage deemed acceptable. Higher moisture levels typically translate into faster degradation of the product; so on average a low moisture level is desired. Typically, less than 1 percent residual moisture is deemed acceptable, although some biologically-based products (monoclonal antibodies) can be damaged at this low level and require a higher residual moisture level for long-term stability.

Typically, samples are pulled from the freeze-dryer at various time points during secondary drying and tested for moisture. A sample thief on the development dryer door is an excellent tool for allowing samples to be removed from the dryer without stopping the cycle and breaking vacuum. Figure 14-43 depicts a development scale freeze-dryer with a sample thief.

Figure 14-43. Sample thief on a development scale freeze-dryer (courtesy SP Scientific)

Lyophilization process development and cycle design

Once the optimal formulation has been developed and the thermal characterization has been completed, it is up to a development scientist, skilled in lyophilization, to develop an optimized cycle for the product. Theoretically, a product can be freeze-dried safely by keeping it as cold as possible (<-40°C); however, freeze-drying is relatively expensive and consumes a large amount of resources, so it is in the best interest of freeze-dried product manufactures to dry them as quickly as possible.

The start of a good, scientifically-based cycle design protocol begins with the collection of the thermal data of the formulation to be freeze-dried. This includes data generated from both the DSC and freeze-dry microscopy experiments. Identifying the type of material (crystalline, glassy, or mixed) and the critical temperatures associated with these different phases is necessary before taking samples to the freeze-dryer for pilot studies.

The first phase of cycle design is freezing the product and conducting an annealing step if necessary. Some products must be kept cold during both formulation and freeze-dryer loading, as they have the ability to degrade quickly. In this case, the shelves would need to be either

pre-chilled or pre-frozen before the product is loaded. Information pertaining to this is generated as part of developing the formulation and should be known before starting lyophilization cycle development.

The product is filled into vials (unless performing bulk tray drying); lyophilization stoppers are partially inserted; and thermocouples are placed into some of the vials to monitor product temperature during development. The vials are loaded onto a tray with a retaining ring then loaded onto the shelf of the freeze-dryer. The special tray is then removed, and the vials are allowed to sit directly on the shelf to optimize the heat transfer from the shelf to the product. The vials must be packed together, and they remain together during the cycle in what is referred to as nesting. Figure 14-44 depicts a typical arrangement of the vials on the shelf. Note that the white circles represent product (or placebo) vials, and the colored circles represent vials that contain thermocouples.

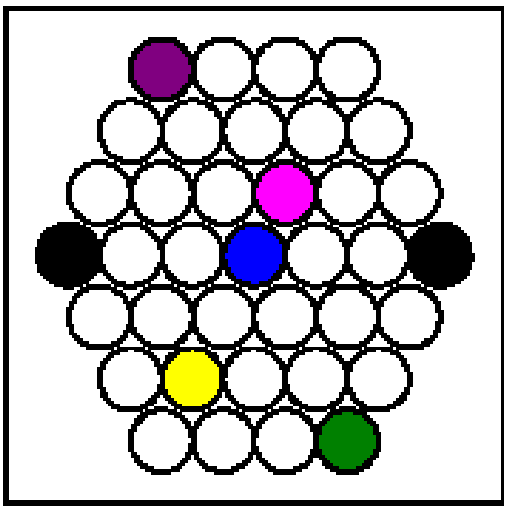


Figure 14-44. Nested vials

The various vial locations are probed with thermocouples in order to understand the temperature differences that result based on the vial's location in the freeze-dryer. Vials in the center experience colder temperature, where vials on the edge see warmer temperatures, as they absorb heat from the walls and door of the freeze-dryer. The samples are frozen, typically to -40°C or lower, to ensure the ice in both the ice channels and the interstitial space is solid. If an annealing step is required to either cause a metastable phase to crystallize or to modify the ice crystal size, then the sample is warmed to the proper temperature (determined by the DSC study) and held for a certain amount of time (typically several hours). The samples are then recooled to -40°C or lower and the vacuum is started.

The vacuum utilized for a cycle is based on two key components—the vapor pressure of ice and the critical temperature of the product determined in the thermal analysis study. For example, if the thermal analysis data showed a critical temperature of -20°C, the product must not be allowed to exceed that temperature until primary drying is complete. In fact, the sample needs

to be kept at approximately 5–7°C below that critical temperature at the start of primary drying, as product temperature rises due to resistance from the dried layer, which is increasing as the samples freeze-dry. For this example, using a critical temperature of -20°C, a starting product temperature (not to be confused with shelf temperature) of -25°C is used. A *vapor pressure over ice* table is then consulted to determine the vapor pressure of ice at -25°C (Table 14-5). According to the table, when the sample is at -25°C (as used in the above example) the vapor pressure of ice at the sublimation front is 476 mTorr. The chamber pressure must be kept lower than the vapor pressure of ice in order for sublimation to proceed at an aggressive rate. Good practice states to keep the vapor pressure in the chamber at 10–30 percent of the vapor pressure of ice at the given temperature. Table 14-5 also includes a column that lists the pressure at 25 percent. So for the above example, if the vapor pressure of the sample is 476 mTorr at -25°C, then the chamber vacuum level should be set to 119 mTorr for optimal drying conditions.

Table 14-5. Vapor pressure over ice values

Ice Temperature (°C)	Vapor Pressure of Ice (mTorr)	25% of Vapor Pressure (mTorr)
-45	54	14
-40	96	24
-35	168	42
-30	286	72
-25	476	119
-20	776	194
-15	1241	310
-10	1950	488
-5	3013	753

Figure 14-45 illustrates the initial conditions for primary drying. It should be noted that the product temperature probes dropped in temperature when the vacuum reached set point. This is due to two events. First, heat travels poorly in a vacuum, so a significant amount of heat from the shelf is lost when attempting to reach the product. Secondly, the act of sublimation is an endothermic reaction, so the product is actually providing itself additional cooling as it dries. At this point product temperature is lower than it should be, so the shelf temperature is warmed in segments until the correct starting product temperature is reached (Figure 14-45).

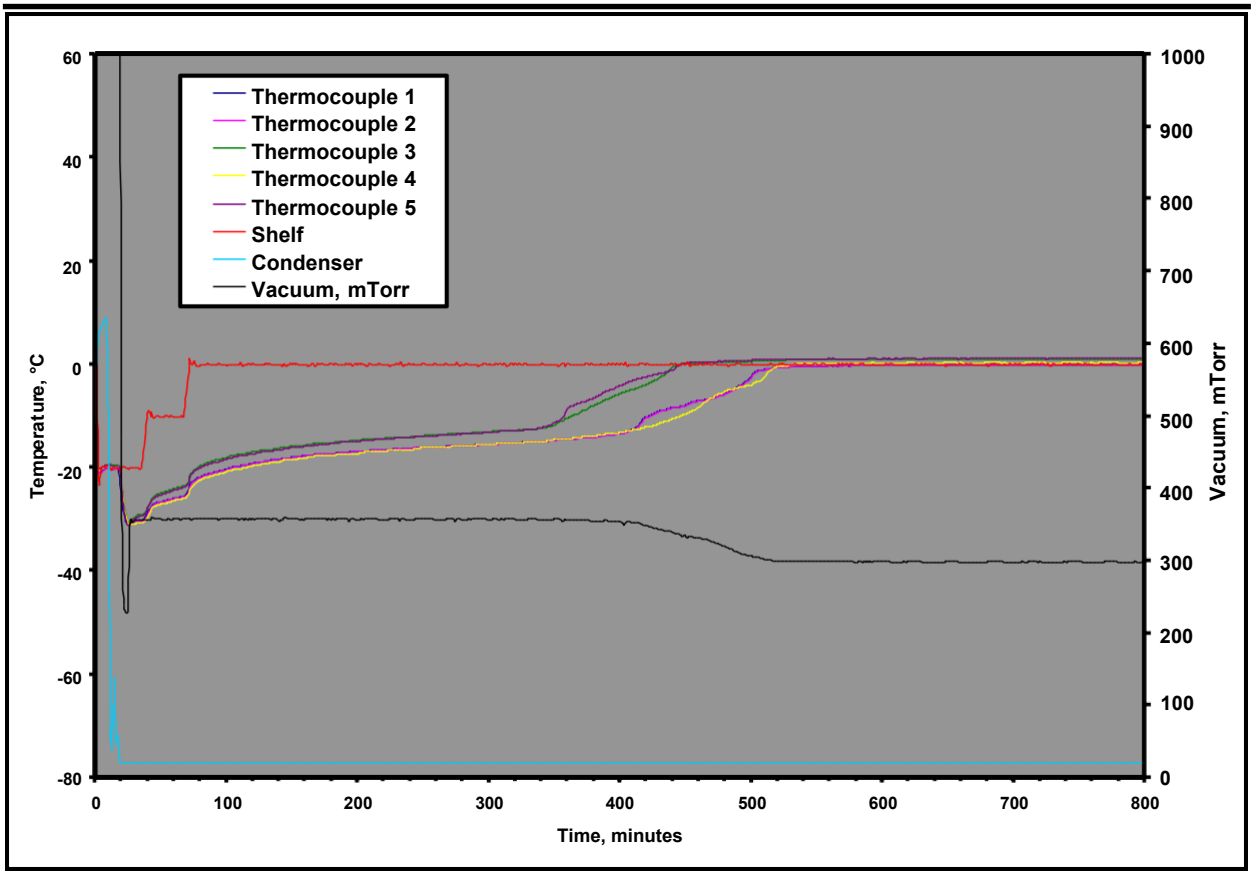


Figure 14-45. Developing the primary drying conditions

At this point the conditions are held constant for primary drying until all of the ice in the ice channels has sublimed. This can be monitored several different ways. First, the product thermocouples show a relatively sharp increase in temperature. This is the result of the sublimation front passing over the tip of the thermocouple. Product temperature rises quickly and levels off near the shelf temperature. This is somewhat limited information, however, as it only provides that of the vials that contain the thermocouples.

The second way in which to monitor the end of primary drying is to observe the behavior of the readout from the Pirani gauge versus the capacitance manometer. The Pirani gauge is skewed by water vapor generated from sublimation. Once primary drying is complete, water vapor is no longer present, and the Pirani gauge returns to reading the correct set point. Figure 14-46 depicts the thermocouples reaching the shelf temperature at approximately the same time that the Pirani gauge pressure reading matches the reading from the capacitance manometer, indicating the completion of primary drying.

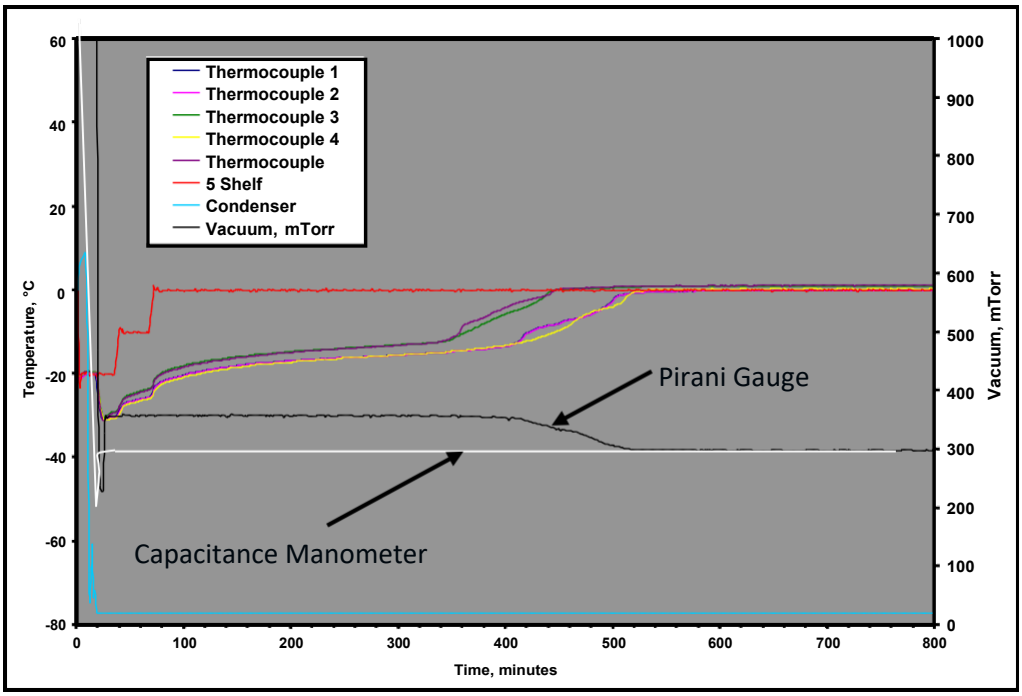


Figure 14-46. Determining the endpoint of primary drying

Another method for determining the endpoint of primary drying is referred to as the pressure rise test. For those freeze-dryers with separate sample and condenser chambers, there usually exists a valve between the two that can be either opened or closed. When the product is known to still contain ice and is active in primary drying, the valve can be closed quickly then reopened. When the valve is closed water vapor is still being created, but since it cannot get to the condenser the pressure rises in the sample chamber. The valve must be reopened quickly during active primary drying or the product becomes too warm and is destroyed. Typically, 10–20 seconds is as long as the valve is allowed to remain closed before reopening. This is usually performed several times during active primary drying in order to obtain a baseline pressure rise value. The valve is closed again when primary drying is thought to be complete, and the pressure rise is then checked. When primary drying is complete there is little pressure rise when the valve is closed; a number of companies use this technique as re-assurance before proceeding to secondary drying.

In secondary drying, the driving force for removing the trace amounts of residual water is temperature. Both the higher the temperature and the longer the product is held at said temperature, the quicker the product drys and reaches a lower moisture level. The temperature is driven by the thermal stability of the product, and the time is driven by how dry the product needs to be. The ramp rate to the secondary drying temperature is driven by the types of materials contained in the product. A completely crystalline material is already mostly dry, so the ramp rate can be extremely aggressive (1°C/minute is considered aggressive). For products containing amorphous phases, the ramp rate has to be slower, as it is possible for a large amount of water to be trapped in the glass, and this water exits gradually. If the ramp rate is too rapid the sample may collapse. Typically a rate of 0.5°C/minute is an acceptable starting

point, but a lower rate may be needed if collapse or cake shrinkage occurs. A typical printout illustrating an aggressive secondary drying phase is depicted in Figure 14-47.

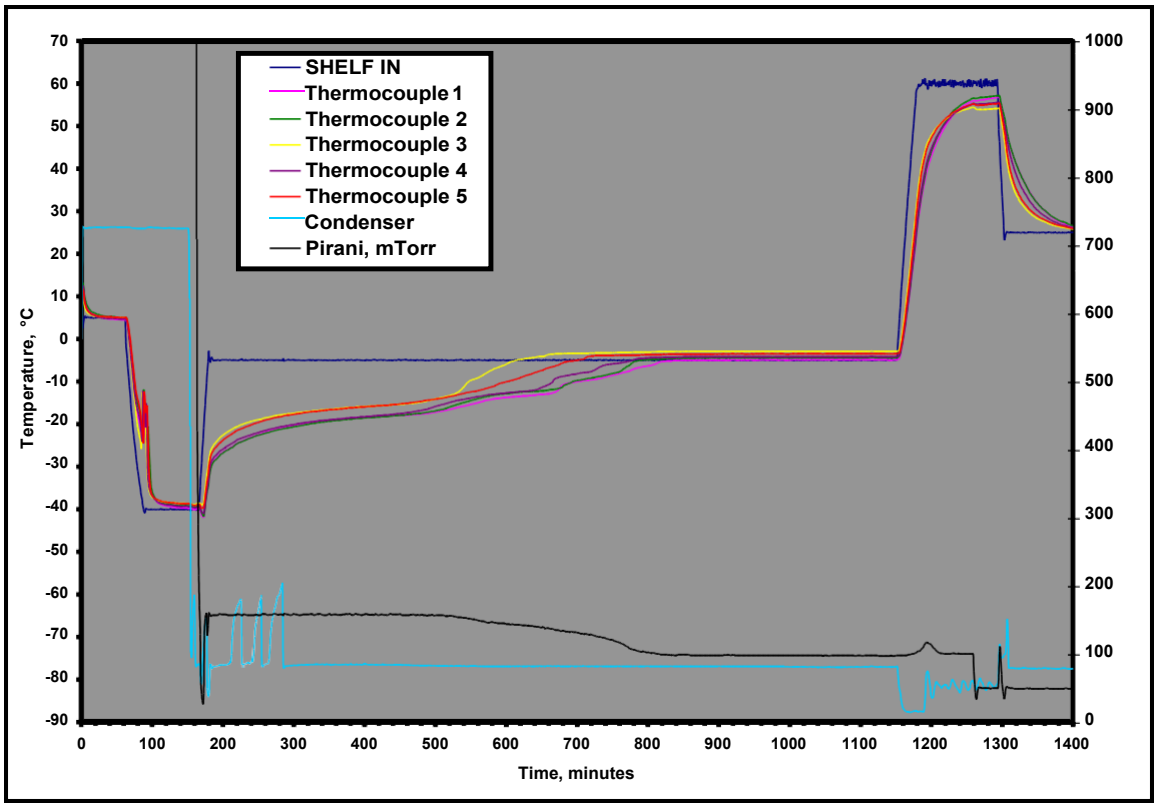


Figure 14-47. Graph of secondary drying phase

The amount of time the samples are kept under primary drying conditions is determined by the amount of residual moisture desired in the product. The only means with which to determine this is to pull samples from the freeze-dryer during secondary drying and measure the water content. Typically this is achieved through a technique known as Karl Fischer (described below); however, the most suitable (and simplest) way to accomplish this is by utilizing an attachment on the freeze-dryer referred to as a sample thief. Samples are typically pulled at the end of primary drying, when the shelves reach secondary drying temperature, and every few hours during secondary drying until the target moisture is achieved.

Pharmaceutical freeze-dryers

Unlike small freeze-dryers utilized for developing lyophilization cycles, those utilized for the manufacture of products that will be either administered to people and animals or used to diagnose diseases all have strict regulations concerning the manner in which they are cleaned, maintained, qualified, and validated. The strictest regulations for pharmaceutical freeze-dryers are for those products being made for human and animal use.

Clean in Place (CIP)

Modern freeze-dryers are manufactured to essentially clean themselves after each cycle in order to wash away any contaminates from the previous batch of drug product. They accomplish this with a combination of spray balls and spray nozzles strategically located throughout the freeze-dryer so that every surface is rinsed with hot WFI or, in some cases, mild detergents. Since all large-scale freeze-dryers are different and built to customer specifications, studies conducted for aiming and locating the different washing devices have to be repeated for every new freeze-dryer.

Before the freeze-dryer leaves the factory, the manufacturer has to verify that the CIP system is working effectively. This is accomplished by spraying a solution of riboflavin throughout the inside of the dryer in order to coat the entire interior surface. The dryer is then run through its CIP cycle; the lights are switched off in the room; and the interior of the dryer is inspected with a UV lamp. Riboflavin glows under UV light, so this procedure makes for an effective means of locating areas that are not effectively cleaned. If traces of riboflavin are discovered, spray nozzles may need to be either repositioned or added and/or spray balls may be needed.

Steam in Place (SIP)

As mentioned above, there are several different ways in which to sterilize the interior of a freeze-dryer. Saturated steam under pressure is by far the most widely used technique, and manufacturers who build freeze-dryers that are to be steam sterilized must take this into consideration when designing these dryers. Due to the high pressure requirements during steam sterilization, manufacturers must design, test, and approve both the sample and condenser chambers as rated pressure vessels. Additionally, there are numerous requirements for other factors, such as pipe length and bends, that engineers must consider, as steam must penetrate all areas of the freeze-dryer in order to serve as an effective sterilizing agent.

The steam sterilization method must be proven to be effective at killing bacteria, molds, and yeasts. In order to accomplish this, biological indicator strips (BI's) are placed throughout the chamber as well as in those areas known to be difficult to reach with steam. BI's are strips that are impregnated with *geobacillus stearothermophilus,* which is a Gram positive, spore-forming bacterium. These types of bacteria, in times of crisis, can become dormant by forming a tough coat around their internal components (a spore), protecting them from the environment. When conditions are more favorable, the bacteria shed the coat and resume their normal life cycle. These types of bacteria are extremely difficult to kill when in the spore state. By verifying that the SIP cycle can fully eliminate these from the freeze-dryer, the manufacturer gains assurance that the CIP cycle is effective at killing any micro-organism. Once the BI strips have been processed through the SIP process, the microbiology group gathers the strips and attempts to grow bacteria from them. If positive growth is observed, the SIP process has failed; changes to the process must be made; and the test must be repeated.

Leak Testing

A leak in the freeze-dryer during a lyophilization cycle is detrimental for a number of reasons. A leak may prevent the correct vacuum level from being achieved during the run, which can affect the way in which the product freeze-dries. More importantly, since the chamber is under

vacuum, a leak means that air is entering the chamber. Since the chamber is sterile from the SIP cycle, the leak allows dirty, contaminated air to enter the chamber and compromise product sterility. Therefore, after completing the SIP cycle and allowing the chamber to cool, the system is sealed (dry and empty), and a low vacuum is created in the freeze-dryer. Once the correct vacuum level is reached, the vacuum pump is switched off and the pressure in the chamber is observed. If the pressure rises beyond a certain predetermined level within a certain time frame, the leak test fails and the task begins to locate and repair the leak.

Qualification (IQ, OQ, PQ)

According to strict FDA guidelines, all freeze-dryer systems, during all phases of installation and operation, must operate in the manner in which they were designed—precisely and accurately. Installation qualification (IQ), operational qualification (OQ), and performance qualification (PQ) are three different phases utilized to prove adherence to proper operation.

The IQ is the first and simplest phase of qualification. This protocol documents that the system was properly installed; that all parts are present; that calibrations are correct and the certifications are in place; that all components are properly assembled; and that all operational manuals, schematics, and other pertinent documentation are accounted for and secured. The results are summarized in an approved IQ report.

The OQ is the most time-consuming and labor-intensive phase of qualifying a freeze-dryer. In this phase the freeze-dryer is essentially put through its paces and operated under several different conditions to ensure it is operating as expected within its operational parameters. Once the IQ is complete and approved, the OQ begins, and is conducted in accordance with approved, specific protocols, with each test documented and verified by operators. The following tests are required to OQ the freeze-dryer, and it should be noted that each of these phases is extremely technical, specifically where they pertain to the placement of temperature and vacuum sensors (location of sensors, manner in which they are placed, etc.). An entire chapter could be devoted to these activities, and pharmaceutical companies employ validation experts to guide and review these activities due to their complex nature. Lyophilization OQ activities include:

- shelf temperature maximum pull down rate
- shelf temperature maximum high and low temperatures
- shelf temperature uniformity (shelf mapping)
- shelf temperature maximum low control temperature during the drying cycle
- condenser temperature maximum pull down rate
- condenser maximum low temperature
- condenser temperature rate capacity
- condenser full load of ice capacity
- vacuum maximum pull down rates
- vacuum maximum low

- vacuum leak rate
- cycle temperature steps, ramps, and holds for temperature (Does the dryer perform as the software instructs it in regards to temperature?)
- cycle pressure steps (Does the dryer perform as the software instructs it in regards to vacuum?)
- stoppering test (Do the shelves compress and completely seal the vials?)
- functionality of all special instrumentation and control routines

Once the freeze-dryer has passed the OQ requirements and the OQ report is approved, the dryer is then qualified to produce injectable drug products. The performance qualification (PQ) is designed to check particular systems should something be changed. For example, if a vacuum pump was replaced, validation would require the section in the OQ protocol targeting the vacuum systems to be repeated—this would be labeled as a PQ study.

Validation

Validation is the process utilized to essentially validate that the freeze-drying process is performing consistently within specifications. To avoid confusion between qualification and validation, a piece of equipment (freeze-dryer) requires qualification (IQ, OQ, PQ), and a process (lyophilization cycle) requires validation.

Validating a lyophilization cycle is a fairly simple process. Basically three batches of drug product are manufactured and freeze-dried. If all three cycles run flawlessly, and each batch tested by QC meets specifications, the process is considered validated. The key is that these three successful batches must be produced consecutively for the process to be truly validated. If these conditions are met, the manufacturer can not only begin producing product to sell but also release and sell the validation batches to the market.

Automated processes and systems

As early as 25 years ago, freeze-dryers for the most part were fully controlled by an operator. It was common practice for companies to keep an employee at the controls of the freeze-dryer twenty-four hours per day and seven days per week while the freeze-dryer was operating. The operator's responsibility was to both record data and manually change the temperature and vacuum as specified in the cycle protocols.

The level of automated control in modern freeze-drying today is staggering. All processes in regards to freeze-drying are now computer-controlled. Not only are temperature, pressure, cleaning, sterilizing, loading, unloading, and stoppering computer-controlled but also all data recording. Freeze-dryers are also capable of utilizing robotic loading systems—computer-controlled transfer carts that transport the vials into the freeze-dryers after they have been filled. Essentially the robot is a large holding tray that is approximately the same size as one of the shelves in the freeze-dryer. A HEPA filter is located above it and provides a Class A environment. The vials come off of the filling line and are packed onto the loading cart. Once full the robotic loading cart follows either a rail or magnetic strip on the floor to the main door of the freeze-dryer. A small door at the bottom of the main door, referred to as a pizza door,

opens and a ram on the transfer cart pushes the vials onto the freeze-dryer shelf. The pizza door then closes and the filled shelf rises, allowing an empty shelf to enter the loading position; the robotic cart then returns to the filling line.

Residual moisture testing

The amount of residual water in a freeze-dried product can have a dramatic impact on both the physical and chemical stability of these products.

Gravimetric

The easiest and simplest way to determine moisture in a dried product is to employ the gravimetric method. According to this method, the sample is weighed on a balance and dried in an oven. As the moisture is then driven off, the vial is reweighed. The difference in weight is the amount of moisture that was in the sample. This method is limited (depending on the sensitivity of the balance) in its detection level of moisture and requires large quantities of sample. This test is also destructive, so the samples must be discarded after use.

Karl Fischer

The Karl Fischer method is by far the most widely used technique and has the highest sensitivity and widest range of detection. This unit can measure water content from the ppm range to 100 percent water. The method uses a chemical reaction with water—the reaction was discovered by Karl Fischer in 1935. The following describes how the Karl Fischer reaction detects and quantifies water:

$$B{\cdot}I_2 + B{\cdot}SO_2 + B + H_2O \rightarrow 2BH^+I^- + BSO_3$$
$$BSO_3 + ROH \rightarrow BH^+ROSO_3^-$$

ROH – Alcohol, typically methanol
B – Base (keeps the pH between 5 and 7, typically imidazole)
I_2 – Iodine
SO^2 – Sulfur dioxide

One mole of water reacts with one mole of Iodine, in which two electrons are released.

There are two different types of Karl Fischer instruments—a volumetric Karl Fischer and a coulometric Karl Fischer (Figure 14-48).

Figure 14-48. Volumetric and coulometric Karl Fischer titrators (courtesy Mettler Toledo)

The systems differ in how they determine moisture. The volumetric is used for larger amounts of water, and the coulometric is used to detect the lower ppm amounts.

The coulometric system is an electrochemical cell comprised of several different components. The anode in the system is a small platinum disc on the end of a glass support rod (also known as the generator electrode), as this is where Iodine is electrochemically created. Directly below this disc is a wire mesh, which is the cathode in the cell. Pulses of current are applied across the anode, and the free iodide ions in the cell are oxidized to iodine. Iodide is clear while iodine is dark brown, so this is observed as small bursts of color around the generating disc. When the iodide is oxidized it releases two electrons, which quickly react with hydrogen ions (H^+), which are, in turn, reduced to hydrogen gas (H^2). Hydrogen gas is observed bubbling from the surface of the cathode during the reaction. A stir bar is located in the cell to both maintain mixing and retain a constant flow of iodide to the iodine generator (anode). When a sample containing water is placed in the cell it reacts with iodine to form iodide ions, so the system continues to generate iodine as long as water is present. When the endpoint is detected (described below) the iodine generation stops and the instrument uses both the amount of current needed and process time to determine the amount of water present.

The cell also contains a double-platinum pin electrode referred to as the measuring electrode. A small current is applied to this electrode, and as long as there is no iodine (I^2) present, a fixed voltage keeps the current constant between the pins of the electrode. Once all of the water is removed there is a trace amount of iodine present, as the water is needed to convert it to

iodide ions. Through a process known as ionic conduction, the voltage required to keep a constant current between the electrode pins drops significantly due to the iodine. The instrument senses this and stops the anode from generating any additional iodine.

The volumetric system operates somewhat differently and does not involve an iodine generator. It instead includes only the detecting platinum electrode. Iodine is pumped into the reaction cell from a reservoir. Higher amounts of iodine are required, as this system is utilized for samples containing larger amounts of water. The sample is introduced into the cell; the cell is mixed with a stir bar; and the pump begins adding iodine to the cell, which is quickly converted to iodide when it reacts with water. Once the water is depleted, the detecting electrode senses this and stops the pump. The system measures the volume of iodine added, and since water and iodine react on a mole-per-mole basis, this volume is the same as that of the water in the sample. As with the gravimetric method, this test is destructive, so the samples must be discarded after use.

Near Infrared (NIR) Spectroscopy

Within the electromagnetic spectrum is a region between 800 nm and 2500 nm known as the near infrared region (NIR). NIR bands originate from overtones and combinations of the fundamental (mid-infrared) bands (mostly from C-H, N-H, and O-H bonds), making FT-NIR spectroscopy especially sensitive to both hydroxyl groups and hydrogen bonding caused by residual moisture. An NIR moisture analyzer is depicted in Figure 14-49.

Figure 14-49. NIR moisture analyzer (courtesy Thermo-Fisher)

A beam within this wavelength range is shot into the sample. The beam penetrates the vial, partially penetrating the dried sample, and is then reflected back to the detector, carrying with

it the information (absorbance) from the sample. Water absorption in this region is strong, so the amount of absorbance is dependent upon the amount of water present. This technique is by far the simplest and fastest water detection method discussed. Additionally, unlike the aforementioned methods, this test is non-destructive, so the samples can be tested and returned to the batch for distribution and sale. The downside of this method is that it must be calibrated, typically by Karl Fischer, before it can be used, as different products behave differently. This makes this method impractical for products prepared once or twice per year; however, this technique is excellent if the same product is produced several times per month.

General Reference

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Check Your Knowledge

1. Which two instruments are typically used to determine the end of primary drying?
   a. CM gauge and thermocouples
   b. Karl Fischer and thermocouples
   c. sample thief and Karl Fischer
   d. Pirani gauge and thermocouples
2. What is the relationship between product temperature and vapor pressure?
   a. exponential
   b. direct
   c. no relationship
   d. inverse
3. Which solid phase entraps more moisture?
   a. eutectic
   b. crystalline
   c. amorphous
   d. lyotropic
4. Why must the product be kept below the critical temperature during freeze-drying?
   a. it will collapse
   b. it will melt
   c. it will dissolve
   d. it will break the vial
5. What are the three typical phases that form in the interstitial space of a frozen solution?
   a. stable glass, liquid crystal, and eutectic
   b. stable glass, metastable glass, and ice
   c. stable glass, metastable glass, and eutectic
   d. ice, metastable glass, and eutectic
6. What occurs if vacuum control is lost and pressure rises?
   a. product temperature rises
   b. product temperature decreases
   c. shelf temperature rises
   d. shelf temperature decreases
7. How is secondary drying shelf temperature determined?
   a. residual moisture of the product
   b. thermal stability of the product
   c. vapor pressure of the product
   d. Pirani gauge reading
8. How is moisture typically measured in freeze-dried products?
   a. Karl Fischer
   b. Pirani gauge
   c. thermocouples

d. pressure rise test

9. How are samples removed from the dryer without stopping the cycle and breaking the vacuum?
   a. vacuum equilibrator
   b. sample thief
   c. pressure relief valve
   d. thermocouples

10. Which two techniques are most widely used for thermal characterization?
    a. DSC and melting point
    b. DSC and freeze-dry microscopy
    c. Karl Fischer and freeze-dry microscopy
    d. none of the above

11. What is the main reason that pharmaceutical products are filtered?
    a. regulatory requirement
    b. to add cost to the prescription drug
    c. to provide a sterile product
    d. none of the above

12. How does a filter remove unwanted particle from a solution?
    a. sieving
    b. entrapment
    c. adsorption
    d. all of the above
    e. none of the above

13. Which are the two most commonly used methods for filter integrity testing?
    a. bubble point
    b. loss on drying
    c. flow loss
    d. diffusive flow

14. Which studies are usually performed during filter validation?
    a. microbial retention
    b. extractables/leachables
    c. filter compatibility
    d. product integrity testing
    e. all of the above

15. What is the main advantage of using either RABS or a Barrier Isolator?
    a. they are inexpensive
    b. they are easy to set up
    c. they require less training
    d. they keep the operator away from the drug product

16. What does hydrophobic mean?
    a. water loving (easily wets with water)

b. water hating (repels water)
c. rabies-inducing
d. none of the above