The AF for minimum dose, which oftentimes is the dose measured at an interior location, is given by

$$AF_{min} = \frac{D_{ref}}{D_{min}} \tag{4}$$

In equation (4), D_{ref} is the dose measured at the reference location and D_{min} is the dose measured at the minimum dose location.

It is important to note that when reference location dosimetry is used to monitor dose during routine processing of product, the minimum dose at an interior location is not measured rather it is calculated on the basis of a statistical relationship given by the AF. For this reason, it is standard practice to measure the dose distribution in more than one product load under the same processing conditions with three product loads considered the minimum number to be dose mapped. Statistical analysis of the data from three dose maps is used to evaluate reproducibility in the measured dose and uncertainty in the statistical relationship that is used to calculate the minimum dose. This estimate of statistical uncertainty in the calculated value of dose can be used to set process parameters for routine irradiation of the product.

Dose Mapping Electron Beam

Because of the much shorter radiation mean-free path of high-energy electrons in materials than high-energy photons and the fact that we are dealing with a beam of electrons, shielding and scattering effects introduced by localized heterogeneities within a carton of product or even within a unit of product in the carton can significantly affect the dose delivered to the product. For example, the range of 10 MeV electrons is approximately 5 cm in water and polymers that commonly serve as packaging materials and closure systems for pharmaceutical products. In a metal such as stainless steel, the range of 10 MeV electrons is less than 1 cm. Therefore, localized high-density regions can result in significant dose gradients within a small volume and shadowing of other regions in the carton of product. These factors need to be taken into account in the selection of the locations of dosimeters within the product load. There are no standard dose map grids as is sometimes the case for gamma or X-ray irradiation. Dose map grids in high-energy electron beam irradiation are unique to each product type. In electron beam irradiation, it is common practice to use reference location dosimetry for monitoring dose during routine processing of product. An external surface such as the surface where the electron beam is incident on the product load may sometimes serve as the reference location or it may be at a fixed location adjacent to the product load and simply referred to as the monitoring location. In the case where the reference location is on an external surface, it sometimes may also represent the minimum dose zone, which would only require use of an AF to calculate the maximum dose delivered to the product load. To establish the reproducibility in dose delivered to the product load and estimate the uncertainty in the AF(s) that is used to calculate dose, multiple product loads, that is, typically three, are dose mapped. The uncertainty in the dose measurement process should be taken into account when setting process parameters.

RADIATION CHEMISTRY

Radiation Interactions with Parenteral Drug Products

As we have seen, high-energy electrons injected into a drug product from a high-power accelerator or generated within the medium from Compton scattering of energetic photons are responsible for the changes in the properties of the drug product and its sterilization. These high-energy electrons, which typically have energies in the 1 to 10 MeV range, suddenly find themselves embedded in the surrounding medium. Atomic electrons of the atoms in the medium effectively shield the attractive force of the positive charges of the nuclei, and the high-energy electrons experience only the repulsive Coulombic force that is instantly established between them. The velocity of a 1 MeV electron is of the order of magnitude 10^{10} cm/sec, which is close to the speed of light. The velocity of atomic electrons is on the order of 100 times less. It takes about 10^{-17} seconds for a 1 MeV electron to cross a diameter of an atom. During that time an atomic electron remains practically stationary and "feels" the rising and falling action of the repulsive Coulombic force created by the approaching and

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leaving of the high-energy electron passing by. The momentum exchanged between the two electrons (the product of the electrostatic force and duration of the collision) is small in comparison with the kinetic energy of the incident electron but may be large in comparison with the binding energy of the orbital electron. If the exchanged energy exceeds the energy that binds the electron to an atom (ionization potential), ionization of that atom will occur, whereas the exchange of a smaller amount of energy will result in its excitation.

Studies have shown that the energy exchange events in liquids and solids involve energy packets between 6 and 100 eV, the most probable being around 25 eV. This is true in simple molecules such as water and cyclohexane (19), as well as in macromolecules such as DNA (20). Obviously all materials consisting of low-Z elements, including biological materials and APIs, absorb energy by similar mechanisms that occur with similar probabilities. The energy of 25 eV is sufficient for the creation of one or two ion pairs and one or two excited molecules in liquid water. The small element of volume within which energy deposition occurs and within which newly formed species are confined for a limited time is called a spur. Occasionally, a larger package of energy is absorbed forming a blob (100 500 eV) or a short sidetrack (500 eV 5 keV). Spurs outnumber blobs by about 50:1 and short tracks by about 500:1. For cobalt-60 gamma rays and 1 MeV electrons in water, the partition of absorbed energy is approximately spurs: 75%, blobs: 12%, and short tracks: 13% (21). Essentially the same distribution of probabilities exists in water vapor and ice underscoring the random character of primary interactions, irrespective of the phase. This leads to the estimate that the absorption of a 1 MeV electron creates about 25,000 spurs, 500 blobs, and 50 short tracks.

The initial volume of a spur in water may be about 1 nm^3 (22), and the volumes of blobs and short tracks may be orders of magnitude larger, 10 and 100 nm^3, respectively. Together they may occupy the volume of the order 10^5 nm^3 containing about 10^6 molecules of water. Sterilization dose of 25 kGy is equivalent to the absorption of 1.56×10^{20} eV/g requiring total absorption of 1.56×10^{14} 1-MeV electrons in 1 g of water. The absorption of this amount of energy would initially affect 1.56×10^{20} molecules/g out of 3.3×10^{22} molecules present in 1 g of water, or 1 in about 200. Allowing that more than 10 water molecules may be contained within a 1 nm^3 spur reduces this estimate to less than one in 2000.

The above picture is oversimplified: there is a distribution of spur sizes and some overlapping of spurs. Nevertheless, it teaches us that precursors of chemical change are initially inhomogeneously distributed only along the tracks of fast electrons while the rest of the volume remains unaffected. It also teaches us that a significant fraction of small molecules may initially escape ionization or excitation, but that larger molecules will not be spared of radiation acting directly. It is also obvious that in solutions, it is mostly solvent molecules that absorb radiation energy resulting in the creation of reactive species. The initially inhomogeneous distribution of primary products: electrons, positive ions, and excited molecules throughout the irradiated medium is one of the key features of radiation chemistry.

Spatial inhomogeneity determines the earliest stage of radiation action, which is termed physical stage. It starts at 10^{-17} seconds with the absorption of energy and extends to approximately 10^{-13} seconds until thermal equilibrium has been reached. The probability of interactions of electronic systems of atoms with photons and electrons during that stage is perfectly random, and nothing can be done to reduce it or to decrease the amount of ionization and excitation. The energy required for the creation of one ion pair in gas (W) is similar (25 30 eV) for a wide range of compounds (23), which forms the basis for the expectation that approximately the same number of ion pairs would initially be created, irrespective of the chemical nature of the substance. However, the amounts of radiation-induced changes that become measurable at later stages greatly differ depending on the medium.

Radiation Chemical Yield

In an empirical approach to quantify and compare chemical effects of irradiation, the measured amounts of radiation-induced chemical changes have been normalized to dose. The quantity obtained in this way is called radiation chemical yield (G):

$$G(X) = \frac{C(X)}{\rho D} \quad (5)$$

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where $G(X)$ is the radiation chemical yield of substance X created, destroyed, or altered; $C(X)$ is the concentration of substance X created, destroyed, or altered; ρ, the density; and D, the dose.

The unit of $G(X)$ is mol/J but an older unit (molecules/100 eV) is still sometimes used (1 mol/J = 9.65×10^6 molecules/100 eV). The knowledge of G values allows the fraction of molecules affected by irradiation of 1 kg of some substance to be estimated as:

$$\frac{C(X)}{C} = 10^{-3} \times G(X) \times D \times M \quad (6)$$

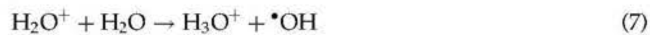
where C is molar concentration of the neat substance and M is its molecular mass. The larger fraction of molecules will be affected by the larger dose and the larger is the molecule. In water, $G(X)$ accounting for all interactions could be on the order of 1 μmol/J, which, for the dose of 25 kGy, gives $C(X)/C = 4.5 \times 10^{-4}$, or about one out of 2000 molecules, which fortuitously well compares with the previous estimate.

If there were no influence of the medium on the initially produced ion pairs, G(ions) in all media would be 100/W, that is 3 4/100 eV (∼0.3 0.4 μmol/J). However, measured values of radiation chemical yields of primary species electrons, ions, and excited molecules strongly depend on the time of measurement and the nature of the medium. This means that they are modified by the medium during the intervening interval of temporal evolution called physicochemical stage that extends from 10^{-13} to 10^{-10} seconds.

Liquid Formulations—Radiolysis of Water

The understanding of physicochemical processes occurring at early stages of radiation action helps in devising meaningful ways to mitigate radiation-induced damage to the parenteral drug product. Parenteral drugs in solid form or a dry state respond rather favorably to radiation. However, liquid formulations particularly those aqueous in nature present more challenges. The peculiarities of aqueous radiation chemistry are discussed in this section.

An important reaction occurring during physicochemical stage in liquid water is the fastest known chemical reaction:



$$H_2O^+ + H_2O \rightarrow H_3O^+ + {}^{\bullet}OH \quad (7)$$

which generates the strongest known oxidizing species, hydroxyl radical. It can oxidize any molecule with which it comes in contact and is mainly responsible for the radiation-induced damage of solutes in irradiated aqueous solutions. Another route for the formation of hydroxyl radical is the dissociation of excited water molecules that becomes possible in the same time window with the onset of molecular vibrations:

$$H_2O^* \rightarrow H^{\bullet} + {}^{\bullet}OH \quad (8)$$

On the same timescale, the reorientation of dipolar molecules leads to the solvation of charged species, notably the free electron becomes hydrated in water, which, as the strongest reducing species known, can affect radiation sterilization of aqueous solutions of reducible substances.

During that time frame radiation-induced species react within spurs or escape from the spurs by diffusion into the bulk where homogeneous distribution of reactive species is eventually established. The recombination of radical species gives stable molecular products:

$$H^{\bullet} + H^{\bullet} \rightarrow H_2 \quad (9)$$

$${}^{\bullet}OH + {}^{\bullet}OH \rightarrow H_2O_2 \quad (10)$$

which, however, are of little concern for radiation sterilization of solutions.

During the physicochemical stage, dielectric properties of the medium have the strongest modifying effect on radiation chemical yields of charged species. Dielectric constant of the medium determines the critical distance at which the Coulombic attractive force of the ion pair equals the thermal energy that drives them apart. Only those electrons that escape the recombination with the parent ion become solvated and eventually participate in the bulk reactions. In a polar liquid like water the probability that an electron will escape the recombination with its parent ion steeply increases with the increase of the initial electron-ion

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separation distance. Therefore, free ion yield is high in water and polar liquids and low in nonpolar liquids.

At the beginning of the chemical stage radiation chemical yields (in μmol/J) are as follows: $G(^{\bullet}OH) = 0.28$, $G(^{\bullet}H) = 0.06$ and $G(e_{aq}^{-}) = 0.27$. Until this moment, the only modifying action on these yields was that of the medium itself, and no additives could have altered them. As it now comes to chemical reactions with the components of the medium, the complex interplay of ionization potentials, electron affinities, bond dissociation energies, and chemical reactivities of the involved species finally determine the outcome of the chemical stage on nanosecond to micro- and millisecond timescales.

The extremely high rate constant of the reaction given by equation (7) and the high molarity of neat water even in concentrated solutions make the reactions given by equations (7) and (8) unavoidable. Any attempts to mitigate in advance ill effects of hydroxyl radical induced oxidations must admit the impossibility to prevent its formation and recognize that the first opportunity to convert it into a more innocuous species occurs only after it has been already formed.

The hydroxyl radical can oxidize any molecule with which it comes in contact and is mainly responsible for radiation-induced damage of solutes in irradiated aqueous solutions. If the substance of interest, an API, reacts with $^{\bullet}OH$ radical with the rate constant k_{API} giving an unwanted product P, it is possible to find a compound S with a preferably higher reactivity with $^{\bullet}OH$ (rate constant k_S), which acts as a scavenger and which does not give P. The hydroxyl radical is thus given two channels to react:

$$\mathrm{API} + {}^{\bullet}\mathrm{OH} \rightarrow \mathrm{P} \quad (11)$$

$$\mathrm{S} + {}^{\bullet}\mathrm{OH} \rightarrow \text{no P} \quad (12)$$

Radiation chemical yield of unwanted product P, G(P) is given by the ratio of probabilities of $^{\bullet}OH$ reacting in the channel giving P to the overall probability of $^{\bullet}OH$ reaction:

$$G(\mathrm{P}) = G({}^{\bullet}\mathrm{OH})k_{API}[\mathrm{API}]/(k_{API}[\mathrm{API}] + k_S[\mathrm{S}]) \quad (13)$$

G(P) will be at minimum the higher the product $k_S[S]$, that is, the more reactive scavenger and the higher its concentration. The same formalism is applicable to all other reactive species.

The hydrated electron and hydrogen atom may be considered a basic and an acidic form, respectively, of a reducing species in the radiolysis of water. Their interconversion is possible because the respective chemical equilibria are strongly shifted to the right. In acidic media, hydrated electrons are converted into $H^{\bullet}$ atoms:

$$e_{aq}^{-} + H_3O^{+} \rightarrow H^{\bullet} + H_2O \quad (14)$$

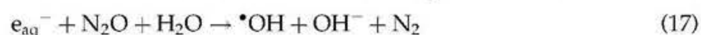
whereas in basic media all $H^{\bullet}$ become e_{aq}^{-}:

$$H^{\bullet} + OH^{-} \rightarrow e_{aq}^{-} + H_2O \quad (15)$$

Using scavengers that specifically react only with the oxidizing or the reducing radicals, it is possible to achieve the presence of only one kind of radicals. In a reducing medium hydroxyl radicals are converted into $H^{\bullet}$ atoms:

$$^{\bullet}OH + H_2 \rightarrow H^{\bullet} + H_2O \quad (16)$$

while in an aqueous solution saturated with N_2O (0.02 mole/L), e_{aq}^{-} are converted into $^{\bullet}OH$:



$$e_{aq}^{-} + N_2O + H_2O \rightarrow {}^{\bullet}OH + OH^{-} + N_2 \quad (17)$$

Tertiary butanol efficiently removes $^{\bullet}OH$ and slowly reacts with $H^{\bullet}$, while other alcohols (e.g., isopropanol) remove both $H^{\bullet}$ and $^{\bullet}OH$. At the same time alcohols do not react with e_{aq}^{-}.

Aqueous (Liquid and Frozen) Parenterals

The absorption of radiation energy in a crystalline solid is not focused on a single atom, but a collective excitation involving many electrons spread throughout the crystal lattice is induced.

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The energy that would have been localized on an individual chemical bond in an isolated molecule in gas or in a molecule in solution is distributed over many bonds in a crystal. Consequently, radiation chemical yield of decomposition in a crystalline matrix is lower than in solution, which is in turn lower than that in gas, $G_{gas} > G_{liquid} > G_{solid}$.

The buildup of free radicals in solids at low doses proceeds proportionally to dose, then the rate of their accumulation decreases until the concentration reaches the limiting value. The limiting concentration is reached when sufficient free radicals are produced within each other's migration volume so that they can recombine. The upper value of the recombination radius critical for permanent trapping in a solid is considered to be about 1 nm (24).

The uptake of radiation energy by a medium is essentially proportional to the total number of electrons (valence and bound) present in a unit volume, that is, proportional to the mass of material exposed to irradiation. On irradiation of solutions most energy is deposited in the solvent. In irradiated aqueous solutions, reactive species e_{aq}^-, $H^\bullet$, and $^\bullet OH$ produced by radiolysis of water react with any dissolved substances that act as their scavengers and consequently suffer chemical changes. Radiation-induced effects that occur as a consequence of the absorption of energy in the target compound are termed direct effects, whereas those that occur in the reactions between a target compound and reactive species produced in a solvent are termed indirect effects.

Effect of Temperature

Direct effects are not expected to depend on temperature. The effects of elevated temperature on chemical reactions of reactive species in solution that are responsible for the indirect effect can be described by the Arrhenius equation. As the activation energies are rather small (6 30 kJ/mol), the effects on reaction rate constants are also not large. The effects of reduced temperature are more dramatic because a significant increase of solution viscosity impedes the diffusion of reactive species, which leads to their spending more time close to their respective places of origin and ultimately, to their enhanced recombination. For example, radiation chemical yield of e_{aq}^- is reduced by a factor of 10 on reducing the temperature from 5 to 55°C (25) and that of the hydroxyl radical by a factor of 60 on reducing the temperature from 20 to 40°C (26). The yields of products derived from electron or hydroxyl radical attack at these temperatures in ice would be reduced by about 90% and 99.7%, respectively, compared to fluid solutions. Because of the reduced mobility at low-temperature reactions, damaging to solute would be possible only at solute concentrations high enough to have solute molecules in a region of reactive species formation, which we have estimated to be one in 2000 water molecules. However, even at low temperature, larger molecules such as proteins cannot escape direct effects.

Effect of Oxygen

Oxygen normally does not react with stable compounds at room temperature, but its paramagnetic properties make it reactive with free radicals, which are also paramagnetic species created by irradiation of APIs, excipients, or solvents:

$$R^\bullet + O_2 \rightarrow ROO^\bullet \quad (18)$$

The most simple route for creating free radicals directly is the dissociation of an excited molecule R-H yielding a hydrogen atom and a free radical residue $R^\bullet$:

$$(R\ \ H)^* \rightarrow R^\bullet + H^\bullet \quad (19)$$

In an indirect radiation action, the abstraction of a hydrogen atom by $H^\bullet$ or $^\bullet OH$ radicals formed in the radiolysis of water or dissociative electron attachment by a molecule R X, containing a strongly electronegative substituent X, also yield free radicals:

$$R\ \ H + {}^\bullet OH \rightarrow R^\bullet + H_2O \quad (20)$$

$$R\ \ X + e_{aq}^- \rightarrow R^\bullet + X^- \quad (21)$$

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Doubly allylic hydrogen atoms, such as found in polyunsaturated fatty acids, are particularly weakly bound to the backbone of a molecule, which makes these locations especially vulnerable to oxidation. Peroxyl-free radicals formed by the reaction given by equation (18) propagate a chain reaction:

$$ROO^{\bullet} + R \quad H \rightarrow ROOH + R^{\bullet} \tag{22}$$

which continue to produce damage of an oxidizable substance as long as there is a steady supply of oxygen.

Oxidation is one of the major causes of drug instability, even without radiation. The ill effects of oxidation can be avoided by the exclusion of oxygen that underscores the importance of packaging and closure systems. It can also be prevented by the use of compounds that interfere with the propagation of radical chains by competing with the reaction given by equation (22), which are known as antioxidants. An antioxidant molecule A H itself possesses a weakly bound hydrogen atom, the abstraction of which produces free radical $A^{\bullet}$, that is more stable (less reactive) than $R^{\bullet}$ and that therefore cannot further propagate the chain reaction:

$$ROO^{\bullet} + A \quad H \rightarrow ROOH + A^{\bullet} \tag{23}$$

More detailed aspects of stabilization of pharmaceuticals to oxidative degradation can be found in (27).

RADIATION EFFECTS

When considering the effects of radiation on a parenteral drug product, it is important to take into account all elements of the drug product that may be exposed to the radiation environment. This includes the container, closure systems, and packaging materials. If the drug product was previously sterilized using a modality other than radiation, some materials that were selected because of physical-chemical features or tribological attributes may not be radiation compatible, which would entail selection of different materials for the radiation sterilization process. Therefore, whenever possible it is important to select the modality of sterilization early in the development of a new drug product.

Container/Closure Systems and Packaging

Most materials that are found in container/closure systems and packaging consist of different types of polymers and glass. In the evaluation of the effects of radiation on these materials, it is important to take into account possible changes in mechanical properties, radiation-induced discoloration, and biocompatibility. Because glass is amorphous, its mechanical properties are unchanged when exposed to radiation. However, most glass materials discolor in varying degrees when exposed to radiation, which may not be acceptable from the standpoint of aesthetics or possibly functional reasons. The degree of discoloration depends on the type and amount of impurities in the glass, which are a source for radiation-induced stable conjugated chromophores. Some types of glass such as cerium oxide glass show less discoloration than borosilicate glass when exposed to radiation (8). A very high purity glass material such as synthetic fused silica also will not discolor when irradiated. Polymers fall into three general classes that include thermoplastics, thermosets, and elastomers. Thermoplastics are the class of polymers that are commonly selected for containment of a drug product, and closure systems are usually elastomeric in nature. A large compendium of information on the effects of radiation on these classes of polymers can be found in published references and from the manufacturers of the polymers themselves (28,29). Only a few polymers are not radiation compatible and should not be used if radiation is the choice for sterilization. Polyacetals, for example, Delrin and Celcon, polytetrafluoroethylene, that is, Teflon, and natural polypropylene are not radiation tolerant and should be avoided. Polypropylene auto-oxidizes and will continue to degrade following irradiation. A radiation-stabilized polypropylene with antioxidants may be used in some applications. Two elastomers that are not radiation tolerant and should be avoided are butyl rubber and a fluoroelastomer. For example, butyl rubber is friable and will shed particulates. It is important to note that a poor choice in the selection of the polymer is not the only reason a part may fail when it is exposed to radiation. Improper

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processing of a polymer or incorrect design may lead to failure of a part that is irradiated even though the polymer is considered radiation compatible. For example, thermoplastics are often fabricated using an injection molding process. If the conditions for fabrication are not optimum, for example, temperature during the mold process, the final part may contain residual tensile stresses. Irradiation leads to breakage of molecular bonds in the polymer. Because of the presence of residual tensile stresses, crazing and microcracking of the polymer may occur. In the design of a part, stress raisers should also be avoided, for example, avoid sharp corners in design of the part.

Radiation Effects—Excipients, Biopolymers, and APIs

Excipients are used to promote pharmacological action of an API by formulation of the drug product in a viable delivery system. Examples of excipients, some of which may appear in parenteral medications, include gum Arabic, talc, starch, and paraffin. The principal effects of radiation that need to be taken into account are change in color, change in pH, and lowering of viscosity. Past studies have shown that excipients should respond favorably up to doses required to sterilize the drug product, that is, 25 kGy or less (30). Loss of viscosity may be of some concern in some cases. In particular, some thickening agents may suffer a significant loss in viscosity at relatively low doses of radiation. Radiation-induced chain scissions in the aliphatic molecular structure of the cellulose component significantly lowers its molecular weight with a concomitant decrease in the viscosity of the thickening agent. Addition of a radical scavenger may significantly improve the radiation stability of the thickening agent.

Biopolymers are used for controlled drug release (CDR) and controlled drug delivery (CDD) of APIs following parentral administration (31). Biopolymers react to radiation in a manner similar to other polymers. There is a possibility of chain scissions, cross-linking, and formation of free radicals. The principal changes of concern from irradiation of biopolymers include change in color and physical properties, which may lead to a change in the drug release characteristics of the biopolymer. For example, polyester polymers such as poly(lactic acid) (PLA) and copolymer poly(lactic acid-co-glycolic acid) (PLGA) are routinely used in CDR/CDD applications. Radiation will reduce the molecular weight of these polymers, with the percentage reduction increasing with increase in absorbed dose. For drug products that have low levels of microbiological contamination, it is possible to set an acceptable minimum dose that satisfies the desired SAL while maintaining a maximum dose that keeps the reduction in molecular weight within acceptable limits.

The principal effects of radiation on an API are formation of small amounts of degradation by-products and possible changes in the chemical-physical properties of the API including pH, color, and viscosity. The radiation-induced degradation by-products may produce toxic extractables that need to be taken into account in the evaluation of the biocompatibility of the API. Changes in the chemical-physical properties of the API could affect the efficacy of the drug product, that is, its potency. Because a vast variety of chemical entities may appear as the APIs, it is almost impossible to accurately predict radiation sensitivity of individual compounds. Previous work on particular or related molecules may inform and guide the assessment of radiation stability of an API.

The effects of irradiation on drugs have been attracting the attention of researchers over the past 60 years. Bibliometric count finds about 1400 references until the year 2000, peaking in the seventies. This literature has been periodically reviewed and a compilation of results from the selection of 217 papers on some 380 APIs has recently been published in form of an encyclopedia (32). Most of the included drugs and excipients are used in sterile product formulations suitable for parenteral administration. The material included in another more recent review (33) is partially overlapping with the former one giving, in addition, an insight into the more recent work, mainly originating from the authors' group. These data may provide clues to the parameters affecting the radiation stability of a drug, the types of possible radiolytic damage, and radiation chemical yields of stable radiolytic products under a variety of irradiation conditions. Together with radiation chemistry principles expounded in the previous section, these data can help the optimization of key parameters to reduce the radiolytic degradation of water-based parenteral drug products. API's in a dry formulation, for

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example, powder or freeze dried, are being successfully terminally sterilized on a commercial level using radiation. Parenteral medications in a liquid form present a greater challenge.

IRRADIATION OF SPECIFIC DRUG PRODUCTS

Vaccines

The use of radiation to inactivate a pathogen in the preparation of a vaccine was explored at an early point in the evolution of the radiation sterilization industry (34). These early studies were typically conducted at relatively high doses of radiation, that is, >25 kGy, which was considered necessary to inactivate the pathogen. Even so, some successes were observed wherein sterility was achieved while the antigenic properties of the vaccine were preserved. Most of these studies appear to only have advanced to a preclinical stage. Over the past several years, there has been a renewed interest in the use of radiation in the preparation of vaccines. The reemergence of certain infectious diseases such as tuberculosis may have stimulated this renewed interest in vaccines that are prepared using irradiation. Dependent on the microorganism, the dose of radiation to inactivate the pathogen may be relatively low. For example, researchers at the University of California, San Diego, have shown that *Listeria monocytogenes*, a bacterial pathogen, was inactivated at doses as low as 6 kGy and the irradiated vaccine still triggered long-term immunity in the vaccinated animals (35). However, viral pathogens, which typically have significantly higher D_{10} values than bacterial pathogens, may require much higher doses of radiation, that is, greater than 25 kGy, to inactivate the pathogen. On the basis of studies that have been conducted over the past several years, a significant advantage of radiation in the preparation of vaccines may reside in the possible formulation of vaccines in a dry state, for example, freeze dried (36). A vaccine that is prepared in this manner could possibly be stored for long periods of time in an unrefrigerated state, shipped world wide to a location of need, and reconstituted on site.

Proteins

Protein drugs are specific, exert their effects at low concentrations, and their virtually limitless number enables their use to influence a large variety of biological processes. Therapeutic proteins include monoclonal antibodies, growth factors, cytokines, soluble receptors, hormones, and proteins that block the function of a variety of infectious agents. Specific functions of proteins in the body strongly depend on their structures.

Proteins are characterized by four levels of structural organization. Primary structure of proteins is defined by the amino acid sequence. The ability of antigenic structures to elicit immune response is mostly a sequence-dependent property. At this (primary) level of structural organization, proteins are rather stable to irradiation. Together with the fact that a considerable degree of denaturation can be tolerated in vaccines, this enables the use of radiation in the preparation of vaccines.

Increasing complexity of structures generally brings about their increased susceptibility to mechanical, thermal, and chemical stresses. Consequently, terminal sterilization techniques, including heat, gas, and radiation, have traditionally not been considered suitable for parenteral solutions of proteins (37). Irradiation of proteins in aqueous solution in the presence of oxygen should be avoided on the basis of the first principles of radiation chemistry because it results in the formation of OH radicals and their subsequent addition to C H bonds along the protein chains, which ultimately leads to oxidative degradation. Irradiation in deoxygenated solutions, on the other hand, favors the reactions of hydrated electrons with peptide bonds and protonated end amino groups. The former reaction also leads to fragmentation and the latter to deamination, and both are unacceptable.

Unique three-dimensional conformation of proteins (tertiary structure) is maintained by the interactions between amino acid residues that are distant from each other in the primary structure. These interactions include hydrophobic and electrostatic interactions, salt bridges, and covalent and hydrogen bonds. They are sensitive to the presence of water, pH, ionic strength and temperature effects, radiation-induced modifications of interacting groups, and dissociation of bonds. For example, an electron adduct radicals formed by irradiation may transfer the electron to a disulphide bond causing its reduction and eventual collapse of the tertiary structure maintained by that bond.

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The weakening of the interactions maintaining tertiary structure at an elevated temperature leads to the loss of the tertiary structure (known as denaturation) of proteins on the one hand and to their increased susceptibility to irradiation on the other hand. For example, a three-time larger reactivity of ribonuclease with the hydrated electron has been observed at 65°C as compared to the reactivity at 55°C (38).

It has been demonstrated that radiation-induced degradation of functional properties of proteins (enzyme activity) in solution can be reduced by reducing the irradiation temperature and by additives. For example, the characteristic e-folding values of doses required for the reduction of an enzyme activity to 37% of its initial value (D_{37} values) could be increased by a factor of 4 if enzymes were irradiated in frozen solutions at 200°C, as compared to irradiation at 30°C (39). Other studies have shown that freezing alone may not be sufficient and addition of antioxidants in combination with irradiation in the frozen state was needed to maintain the integrity of the protein at high doses, for example, 50 kGy (40). Even at low doses, for example, 10 kGy, in a low pH solution, irradiated insulin suffered significant cleavage, dimerization, and oxidation (41). Addition of scavengers such as ascorbic acid or oxidized glutathione along with processing at dry ice temperature provided sufficient protection to enable recovery of more than 90% activity. It should be possible to irradiate proteins at dry ice temperature on a commercial level without major constraints. For example, tissue products are being routinely irradiated at dry ice temperature. Irradiation at lower temperatures, for example, liquid nitrogen temperature, would prove more difficult.

However, lyophilization with a well-designed formulation should enable irradiation sterilization to be utilized for terminal sterilization of the drug product. Drying reduces the secondary or indirect damaging effects from radiation while allowing the primary effect to inactivate the pathogens for the desired SAL. Suppression of secondary effects requires the addition of radical scavengers, for example, hindered phenols, ascorbic acid, cysteine, and glutathione (42 45).

Published information on the effects of dose rate on response of irradiated proteins does not provide a clear answer as to whether high-dose rates or low-dose rates are preferred. It is possible that dependence of temperature change on dose rate has clouded some of the results. All other factors being equal, higher dose rates will typically lead to a larger increase in the temperature of the irradiated product. Because temperature can play an important role on the response of proteins, it may be responsible for observed deleterious effects on irradiated proteins rather than dose rate.

CONCLUSIONS

Since the 1950s, radiation has been used to terminally sterilize a whole host of health care products and many types of pharmaceutical products including those used in parenteral medications. Radiation sterilization is an efficacious process that is simple to apply and can be validated in a straightforward manner using existing methodologies. International standards offer guidance on execution of the process. Today 170 gamma irradiators and 41 electron beam irradiators are being used around the world for commercial sterilization applications. Only a few X-ray irradiators are presently operational, but that will change with time. High-energy electrons from high-power accelerators, gamma rays from radioisotopic sources, and X-rays from accelerator-initiated sources are all capable of penetrating deeply into most materials, thus effectively sterilizing all elements of the product. Investigations have shown no evidence of nosocomial infections that are traceable to the sterilization process, for example, Epidemiologic Investigations by CDC/Hospital Infections Program, 1980 to 1990.

This chapter has attempted to elucidate all the key features of the radiation sterilization process including methods of controlling the environment to the benefit of the product that is being irradiated. The section on radiation chemistry focused on liquids, which represent a greater challenge to the radiation sterilization process than drug products that are formulated in a dry state. Dry formulations of parenteral drug products are presently being successfully radiation sterilized on a commercial basis. Methods for improving the tolerance of liquid-based parenteral medications to radiation including addition of anti-oxidants or scavengers and irradiation in a frozen state are presented in this chapter. Because the radiation dose to achieve a desired SAL is bioburden based, the anticipated very low bioburden of liquid-based

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parenteral drug products should allow their sterilization at low doses of radiation, thereby enhancing the probability of success. With the development of new biologically derived drugs and combination drug-device products, there will be challenges for effective sterilization of these products. Radiation may become a preferred modality for terminal sterilization of many of these complex products.

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13 Filters and filtration

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FILTRATIVE SEPARATION

Sterile filtration is widely used in the biopharmaceutical industry to remove contaminants, especially microorganisms from liquids and gases. Microorganism removal is required either to achieve a sterile filtrate or to reduce bioburden levels, which in turn decreases endotoxin threats. Sterilizing grade membrane filters are defined by the FDA Guideline on Sterile Drug Products Produced by Aseptic Processing, 2004 by being able to retain more than 10^7 *Brevundimonas diminuta* (formerly *Pseudomonas diminuta*) organisms per cm^2 of filtration area at a differential pressure of 29 psi (2 bar). The retention efficiency has to be validated, using the actual drug product and the process parameters, because of the possibility of an effect to the filters compatibility and stability and/or the microorganism size and survival rate. Performing product bacteria challenge tests became a regulatory demand in 1995 and is now a part of standard filter validation (1). Prior to performing challenge tests, the appropriate challenge methodology has to be evaluated via viability tests. These tests determine the mortality rate of the challenge organisms due to product or process parameters. PDA Technical Report No. 26, 2008, describes the individual parameters, the possible effects, and mechanisms to be used to perform challenge tests. Additionally, the report discusses filtration modes, sterilization, and integrity testing. Multiple parts of this document have also been adopted by ISO (2) and the FDA Aseptic Guideline (3).

FILTRATION GOALS

Contamination Removal

Prime purpose of filtration is the removal of contaminants, which however can vary either being particulates, microbial, viral, colloidal, or gels, etc. The first essential question to be asked when filtration steps are developed or implemented would be what the retention purpose is? The answer to this question is the basis for any step and decision that follows. For every application and removal purpose filter types and designs are required to reach an optimal result. Retention mechanisms of the various contaminants differ, the filter construction can be affected by the contamination type, and the performance of a filter is determined by the form and load of the contaminant (4).

For example, colloidal contaminants, haze, or lipids are retained best by adsorptive filter forms (see adsorptive separation). Microbial retentive filters, especially sterilizing grade filters, should preferably be sieve retentive to assure appropriate organism removal and filtrate sterility. Pore-size specification depends on the contaminant removal purpose, as a filter that is too tight could result in performance losses or oversized systems. Most of the time the purpose of filtration is to remove the contaminant but pass the drug of interest through the filter. Again, a membrane that would be sized too tight could jeopardize the yield outcome. The removal need requires to be well defined and should fit the filtrate quality necessity without dismissal of filtration performance criteria (5).

The design or construction of the filter is determined by the removal need and contamination load. If the load is high and the particulate matter size distribution widespread, the filter should be designed to gain fractionate retention, meaning larger particles are retained first and smaller gradually within the depth of the filter. Such filter would have a multilayer construction to cope with the load and spectrum. If the contaminant is well defined, a sharper retentivity can be utilized, and it may well be that only a single layer membrane will be able to separate the target contaminant. The design of a filter element and/or a filter combination depends on the contamination form and load.

Process parameters, especially differential pressure conditions, often require careful observation, as an elevated starting differential pressure could block the filter prematurely, especially with gel-like contaminations. Filter cake compaction in such instances has to be

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avoided as the total throughput would be restricted. The pressure conditions in these contamination occurrences require being as low as possible to avoid any premature blocking.

Rate of Flow

Flow rate becomes a main focus when the fluid to be filtered has a limited amount of contaminants or fouling components. In this instance, a particular fluid volume must be filtered in the fastest time possible. Important here is the timeframe of the filtration process to make the equipment available again for reuse as fast as possible, as it determines parts of the downtime and therefore the capacity available within a production facility. For example, a low flow rate 0.2 μm filter (2500 L/hr) would require 48 minutes to filter a 2000-L volume versus only 20 minutes for a high-flow filter (6000 L/hr). This would reduce equipment use time by half or the effective filtration area (EFA) could be reduced, which would cut filter costs. High flow rates are most commonly required in the filtration of buffers or large volume parenterals. To gain optimal flow rates from membrane filters, there are limited parameters that can be controlled within the filtration process. Either the differential pressure, which is limited, can be raised or larger filter surface can be applied, with the disadvantage of increases in consumable and capital investment costs.

Flow rate depends on the whole filter cartridge design and not solely on the membrane's porosity, thickness, and construction. If a membrane, with an exceptional flow rate, cannot be pleated, it is of no use within a filter cartridge construction. The flow rate optimization of filtration processes requires tests using comparable filter elements, commonly 10 in. filter cartridges. A side-by-side trial can be performed using such comparable filter units, as only this test method would evaluated the entire design of the filter and membrane, in addition to the EFA, flow distribution due to pleat densities, and the fleece thickness. The test would be performed under the specified process conditions, commonly using a set inlet pressure, while the time to filter the fixed fluid volume will be measured. Important is that the process parameters are kept constant, meaning the same buffer composition, pressure, and temperature settings must be applied.

The use of 47 mm disks as an indicator trial for flow rate is meaningless, as these tests do not take constructive designs into consideration. Forty-seven millimeter flow rate results differ greatly from the 10 in. element flows and are of no use in determining an appropriate filter type and scale. Only large-scale trials can determine the best flow rate filter (6).

Total Throughput

Total throughput, meaning the total volume filtered before the filter element blocks, is probably the most widely required performance criteria in most filtrative applications. It is directly proportional to the filter design, surface area, system size, and prefilter combinations. Total throughput has a major impact on filtration costs, and what might appear to be a less expensive filter may actually significantly increase the filtration costs.

The total throughput of a filter cartridge depends on the membrane filter polymer, pore structure, and filter design. Some membrane polymers are adsorptive and higher adsorptivity is commonly associated with a higher fouling rate and therefore lower total throughput. Membranes with a higher asymmetric proportion, meaning a larger pore structure on the upstream side than filtrate side, commonly have a larger total throughput than a symmetric membrane structure due to fractionate retention. Another design improvement is the membrane combination within the filter element. A coarser prefilter membrane layer in front of the final filter membrane, the so-called heterogeneous double-layer membrane, has a distinctly higher total throughput.

Throughput is also referred to as the filter's capacity, meaning the filter's capacity for dirt-holding. It can be expressed as the mass of particulate matter held by a filter, or by the volume of fluid supplied to a filter, it being assumed that the dirt concentration of the suspension is homogenous and constant. The capacity of a filter is a measure of the total volume of fluid that can be processed before a pressure drop develops to decrease the flow to an unacceptably low rate. Capacity may vary dramatically depending on the particles' types and sizes, whether hard and incompressible or gel-like and deformable. The goal aimed for is

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the complete and timely processing of a production run; timely being defined in terms of practical and economic significance (6,7).

Throughputs can be judged insufficient; if given the selected conditions of available filter area and differential pressure, the quantity of effluent produced is at too slow a rate to meet the time requirements of the operation. The correction of the condition can be managed by increasing the filter's available area, the differential pressure's (Δp) driving pressure, or both. The larger ratio of EFA to TSS (total suspended solids) thus contrived may limit the filter cake build-up to more modest depths over the filter's now-larger surface. Less blockage would result per unit time. Increasing the filter's available area, or the Δp's driving pressure, or both should correct this condition. If the filtration is already under way, increasing the filter area will be the more difficult alternative. Raising the differential pressure, although easier to accomplish, deserves judicious application. It risks compaction of the retained particles and reduction of the flow rate.

The total throughput can be further advanced by evaluations of appropriate pre- and final-filter combinations, if required. A lower-cost prefilter might be used to protect the final filter and reduce the required final filter size.

Total throughput tests to determine the appropriate final filter and/or combination of pre- and final filter are performed with 47-mm flat filter composites. These composites have to have the same fleece and filter combination as the filter element to be used later. Commonly, multiple composites are tested to determine the appropriate final filter and to be able to test multiple prefilter options. These tests will determine the optimal combination that achieves the highest fluid throughput per EFA.

Nevertheless, 47 mm tests can only suggest the best filter combination. To define the proper filter size required within the production process, small-scale pleated devices of the predetermined filter combination should be utilized.

Unspecific Adsorption

Unspecific adsorption is the second leading cause of yield loss within biopharmaceutical processes after protein degradation due to slow process flows. Any yield loss is proportional to loss of production capacity and market value. Therefore, unspecific adsorption testing must be a priority within applications, which might be adsorption sensitive. Applications encompassing drug products containing preservatives and therapeutic proteins are common examples of adsorption-sensitive processes.

Protein adsorption is a many-faceted phenomenon. It is difficult to predict. Protein surfaces can contain different hydrophobicity, charge, and degree of hydration, and can change with protein conformation and with solution characteristics (8). The filter surface has similar differences. Neither surface is uniform regarding charge or composition. Both hydrophilic and hydrophobic adsorptions may occur. The interaction of protein and surface increases with the hydrophobicity of each. Therefore, hydrophobic adsorptions are believed to reflect protein-filter interactions.

Truskey et al. (9) measured protein adsorption, circular dichroism, and the biological activity of protein solutions. Shifts in circular dichroism and decreases in enzyme activity resulted from conformational changes of the protein structure. Protein-membrane interaction caused the protein to expose its hydrophobic sites, which were folded within its structure during its exposure to aqueous solution. This shows a connection between protein shape and function. Also, shearing of protein molecules and loss of protein properties may result from passage through a filter's tortuous pores. But shearing is seen as causing fewer functional losses via denaturation than do adsorptive conformations.

As described in testing for total throughput, commonly unspecific adsorption assays are performed during small-scale trials or within the process validation procedures of a filter into the specific product and process specifications. Small-scale trials should be performed as early as possible to avoid any surprises or possible validation delays further down the development process. As these trials commonly utilize a small volume of the actual drug product, optimization trials can also be performed. For example, in certain applications it has been found that buffer flush, specific pH, or temperature conditions can minimize the unspecific adsorption into the filter membrane. These conditions require evaluation besides the actual

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membrane filter polymer and composite. Forty-seven millimeter disk trials, as in total throughput evaluation, are the best evaluation tool to find the most favorable process parameters and membrane polymers, but the unspecific adsorption of a filter element is also directly proportional to the EFA and the design. The larger the area or the more membrane layers that are utilized the higher the adsorption.

TYPES OF FILTERS

Membrane Filters

Membrane filters commonly have a defined pore structure and porosity band. The narrower the porosity band the more defined the retention rate of such membrane is. The filtration obtained by the use of such membrane filters is often referred to as microfiltration, or MF. Microporous membrane filters have a much-defined porosity than is available within prefilter matrixes. Depth filters have a randomness of the fibrous material that does not allow producing a defined porous structure as within membrane filtration. Membranes are produced by an evaporation, quenching, stretching, or track-etched process. In the evaporation process, the casting solution is applied onto a belt. Because of defined temperature, belt speed, and air conditions, the solvent from the casting solution starts evaporating, this leads ultimately to the formation of the wet-gel form of the microporous membrane (10). Changes in the described conditions and the casting solution mix will create different pore structures, porosities, and membrane structures. In the quenching process, the polymer/solvent mix is applied onto a drum or belt, which immerses into a solvent or extraction bath. The polymer dope starts precipitating and forms a porous membrane. Stretching production process to form membrane is mainly used for polytetrafluoroethylene membranes. Melt extruded films are stretched under defined process conditions to create a thin membrane. The thinnest (10 20 μm) membrane films are created by track-etched manufacturing process. Commonly polycarbonate is subjected to a barrage of high-energy particles. The membrane polymer is damaged at the particle track, and after the submittal to an etching bath pores are formed along the damage. The pore structure of track edge membranes is very defined, but due to the avoidance of particle track overlaps, the porosity is low.

Membrane filters can be formed in a variety of structures for specific application purposes. For example, the formation of asymmetric membrane structures, the pore size on the upstream side is larger than the downstream side of the membrane, can enhance the dirt load capacity of such filter. Membrane filters are the most common filtration devices used in aseptic processing to remove organisms from liquids or gases. Because of the defined structure, these filters are highly reliable with respect to the retention requirements and furthermore can be integrity tested.

Depth Filters

Lenticular filter designs are mainly used as clarifying filters. Highly adsorptive cellulosic or kieselguhr-containing depth filter pads are welded together in a plate format. These plate formats commonly have a diameter of 12 or 16 in. and contain stacks of 4 to 16 to create a depth filter unit. The benefit of lenticular filter types is the high dirt load capacity. The adsorptive depth filter material is ideal to separated colloidal substances and lipids; as a result these filters are very often used in plasma and serum applications. Nowadays lenticular filters are most often used in cell harvest applications after the fermentation process.

When lenticular filter combinations are tested, the tests do not only involve the total throughput of the filter element as it is commonly the case with pleated prefilter cartridges, but an important factor is the turbidity measurement of the filtrate. The turbidity measurement will create an indication of the protective properties of the lenticular filter retention rating used and how much of the contaminants are separated by the particular filter rating.

Test methods, validation requirements, and design specifics have recently been elaborated in the PDA Technical Report 45, Filtration of Liquids Using Cellulose-Based Depth Filters (11).

Prefilters

Prefilters are most commonly depth filter types and are generally constructed of nonwoven or melt-blown fiber materials such as polypropylene, polyamide, cellulosic, glass fiber, metal

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fibers, and sintered stainless steel (12). Most commonly, prefilter materials are constructed into mats by the random deposition of either individual or continuous fibers whose fixation is accomplished by pressing, heating, gluing, entanglements, or other forms. The pores of such filter constructions are rather random interstices among the fibers. Such pore-size distribution can be influenced by the thickness of the individual fiber or the compactness of the matrix. Therefore, prefilter types have a large variety and can be selected for many kinds of application.

A major advance in depth filter design technology was the construction of melt-spun depth filter types and the introduction of heat stabilization of fiber fleeces. These treatments avoided the release of particulate matter and were utilized to stabilize the final filter fleece. Additionally, these technologies allowed producing fleece construction of different fiber diameters within a filter matrix. This allowed improving the total throughput performance of these filters due to fractionate retention of a large spectrum of particle sizes. A further advance in depth filter technology occurred with the advent of the first melt-blown type of cartridge that incorporated various fiber diameters, as the filter was manufactured, to achieve a graded pore design by means other than varying the fiber packing density. This design is based on using a variation of standard melt-blowing equipment. In this process, the polymer is extruded through a multihole die and the polymer stream is stretched and attenuated by a high-velocity heated air stream. The mean fiber diameter is changed as the filter is being made by adjusting the air velocity or one of the other variables that contribute to the formation of the fiber sizes, for example, temperature or polymer pumping rate. This technology is becoming more advanced, with some manufacturers naming the fibrous fleece constructions as nanofiber fleeces.

The concept of using a graded or changing pore size to enhance filtration performance is a desirable one. This technique involves incorporating a series of prefilters into a single stage to maximize the use of the entire filter and extend filter life (dirt-holding capacity). The factor of fractionate retention is especially important for applications with a wide particulate spectrum, as for example water pretreatment.

Prefilters can also contain membranes, porous or fibrous, commonly from cellulose, mixesters, or borosilicate. These prefilter types are utilized to remove a very fine band of particulate or contaminants from the fluid to specifically protect sterilizing grade membrane filters. The retention rating of a prefilter is not defined by pore size but by nominal retentivity, commonly a particle-size retention of more than 99%.

Nanofilters

Most commonly, nanofilters are designed to separate viruses, using size exclusion as the predominate mechanism of removal. Since nanofilters are extremely tight filters, the water bubble point is commonly higher than the maximum allowable operating pressure. Therefore, integrity testing of these filters requires special test methodologies, such as liquid porosimetry (13,14). This test method uses two immiscible liquids that are successively intruded by pressure into largest pores of the membrane. These porosimetry measurements may be correlated to viral removal post filtration, which allows the test to be used to validate viral removal in actual practice. Nanofilters or viral retentive filters are an essential contaminant removal step especially in bioprocesses. A multitude of nanofilters are available for different applications and target contaminants. Most common retention ratings are 20 and 50 nm, also known to separate parvo- and retroviruses.

GENESIS OF PORES

Pore Structure

Microporous membrane's analogy is that of a polymeric sponge (Fig. 1). The oversimplified picture of the filter pores is that of irregular and tortuous capillaries composed of the interconnected spaces within the polymer matrix. The structure derives from a polymer solution and the chain segments are separated from one another by distances that reflect the polymer dilution. It is the final interstitial distances that in their interconnections prefigure the pores of the finished membrane. Formulae of different polymer concentrations give rise to different intersegmental separations, ultimately to different porosities (15).

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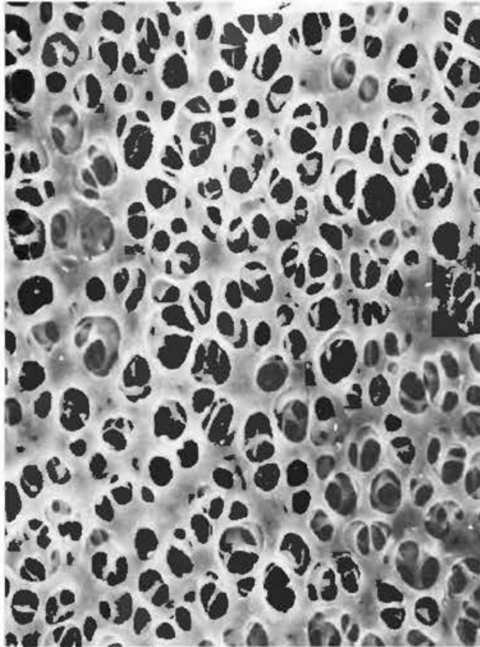

Figure 1 Microporous membrane structure. *Source*: Courtesy of Sartorius Stedim GmbH.

The casting solution consists of polymer dissolved in a mixture of solvent and high-boiling nonsolvent. Pore formation occurs as follows: As solvents progressively evaporate from the casting solution, the nonsolvent increases in content to the point where phase separation takes place. Nonsolvent droplets separate within the polymer/solvent phase, and polymer comes out of solution to concentrate at the droplet interfaces. The swollen polymer shells surrounding the nonsolvent droplets thicken as continuing solvent loss causes more polymer deposition. The eventual disappearance of the polymer/solvent phase brings the polymer-surrounded droplets into mutual contact. They consolidate into clusters, and distort into polyhedral cells filled with nonsolvent under the impetus of the area minimizing forces. Finally, the edges of the cells accumulate polymer at the expense of the cell walls. Thinning of the walls of the polyhedra leads to their rupture and interconnection. The reticulation of the discrete cells of the polymeric matrix permits the removal of the nonsolvent, as by washing. Not the polyhedral cells, but their interconnecting openings, thus formed, comprise the metering pores of the membrane (15).

Polymeric Types and Properties

As one can expect, there are distinct differences between the individual membrane and prefilter polymers. Table 1 lists the different membrane polymers available and the advantages and disadvantages, which depend on the properties of the polymer. The table shows that there is no such thing as a membrane polymer for every application. Therefore, filter membranes and the filter performance have to be tested before choosing the appropriate filter element.

PORES SIZE

Ratings

Where sieve retention of particles is the only consideration, the size of the largest pore, present in the filter is ultimate concern. Particularly in the filtrative sterilizations of pharmaceutical preparations, there is an emphasis on achievement of that particle size/pore size relationship that can produce organism removal solely by sieve retention. However, in theory, complete organism (particle) removal does not require the exercise of sieve retention. Adsorptive particle capture may also be utilized. Microporous membranes are used in filtration sterilization because there is considerable surety of particle retention that can be in most

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Table 1 Properties of Different Membrane Polymers

Membrane material	Advantage	Disadvantage
Cellulose acetate	• Very low nonspecific adsorption (nonfouling) • High flow rates and total throughputs • Low environmental impact after disposal	• Limited pH compatibility • Not dry autoclavable
Cellulose nitrate (nitrocellulose)	• Good flow rate and total throughputs • Capture of smaller particles than the pore size	• High nonspecific adsorption • Limited pH compatibility • Not dry autoclavable
Regenerated cellulose	• Very low nonspecific adsorption (nonfouling) • Very high flow rates and total throughputs	• Limited pH compatibility • Not dry autoclavable
Modified regenerated cellulose	• Very low nonspecific adsorption (nonfouling) • Moderate flow rates and total throughputs especially with difficult to filter solutions • Broad pH compatibility • Easily cleanable (in cross flow applications required)	• Ultrafilters not dry autoclavable
Polyamide	• Good solvent compatibility • Good mechanical strength • Broad pH compatibility • Dry autoclavable	• High nonspecific protein adsorption • Low hot water resistance • Moderate flow rate and total throughput • Vacuole formation during casting can result in exaggerated pore sizes
Polycarbonate	• Good chemical compatibility	• Moderate flow rates • Low total throughputs • Difficult to produce
Polyethersulfon	• High flow rates and total throughputs • Broad pH compatibility • Highest versatility • Mainly found as asymmetric membrane structure	• Low to moderate unspecific adsorp tion depending on surface modifica tions • Limited solvent compatibility
Polypropylene	• Excellent chemical resistance • High mechanical resistance	• Hydrophobic material • High nonspecific adsorption due to hydrophobic interactions
Polysulfone	• High flow rates and total throughputs • Broad pH compatibility	• Moderate to high nonspecific adsorption • Limited solvent compatibility
Polytetrafluorethylene	• Excellent chemical resistance • High mechanical resistance • High hydrophobicity (used for air filtration)	• Hydrophobic material • High nonspecific adsorption due to hydrophobic interactions • High cost filter material
Polyvinylidene difluoride	• Low nonspecific adsorption • Dry autoclavable • Good solvent compatibility	• Moderate flow rate and total throughput • Hydrophobic base, made hydrophilic by chemical surface treatment; may lose hydrophilic modification due to chemical attack • High cost filter material

cases demonstrated to be independent of operating conditions. Sterilizing grade membranes are expected to have a pore-size distribution pattern wherein the largest pore is smaller than the smallest microbe whose retention is being required. Sieve retention is consequently assumed to be the sole particle-capture mechanism operational. This is the intended situation, for the dependability of sieve retention is seen in its freedom from the operational factors that

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influence the efficiencies of adsorptive removals, such as the organism challenge level, the magnitude of the applied differential pressure, and even such parameters as fluid temperature, viscosity, ionic strengths, the presence of wetting agents, etc., that constitute the contribution of the liquid vehicle (16,17). In fact, filter reliability, involving whatever mechanisms of particle removal, is demonstrated beyond doubt by the exercise of filter validation.

Semantics enter the picture of the largest pore. As commonly considered, a penetrating particle encountering the filter enters by way of a large enough pore and completes its penetration unhindered. In this scenario, the large inviting pore maintains its generous dimensions clear through the filter. In this sense, the bubble point assay measures the diameter of the entire pore passageway; no distinction is made between the "largest pore" and any particle-restraining portion of the pore. Actually, the pore diameter not being uniform throughout the bubble point measures the narrowest point of the overall widest pore.

Regrettably, the current use of the word "pore" is undifferentiated with regard to its meaning. Its use covers both the polyhedral chambers and their connecting, restrictive, smaller apertures. The pore passageways consist of an assemblage of larger and smaller apertures interconnecting the polyhedra. Overall, certain of these passageways are the largest in the sense that they are least restrictive. However large the passageways, it is their restrictive dimension that is measured by the bubble point. In this sense it is not the largest pore, the largest aperture leading from the polyhedron, but the narrowest of those comprising the largest pore path overall that comes to be measured. Strictly speaking, therefore, it is not the largest pores that are revealed in the bubble point measurement but the most restrictive ones associated with them in the overall largest pore path.

Distribution

Pore-size distribution means the spread of different pore sizes within the membrane structure. The membrane structure being sponge like, one finds a pore size spread over the entire membrane structure, width as well as depth. The efficiency of particle removal varies inversely with the challenge density. This can be explained on the basis of a pore-size distribution wherein the number of smaller pores far outweighs the fewer large pores. Only when so great a number of organisms are present as to enable confrontations with the few larger pores, might organisms escape capture. The attention, especially in sterilizing filtrations, is so focused on restraining bacterial passage that only the largest pores, those that the organisms can negotiate on a size basis, are a matter of concern. Hence, the emphasis is on the bubble point measurement of the set of largest pores. There is reason to believe that, despite their relative paucity, the larger pores are early on engaged by the hydrodynamic flow when diluter organism suspensions are fed to the filter (18 20). One factor that had delayed explanation of the dependence of organism retention on the challenge density was the de-emphasis of the pore-size distribution. The pore-size distribution of membranes had early on been explored by mercury porosimetry.

When mercury is forced into a pore, the pressure required to fill that pore completely is in inverse proportion to its size. The relationship is, as for the capillary rise equation,

$$D = \frac{4\gamma \cos\theta}{P}$$

except that the minus sign is required by the nonwetting nature of mercury relative to membrane surfaces. Here P is the pressure; D, the pore radius; γ, the surface tension of mercury; and θ, the contact angle of mercury with the solid pore surfaces.

Assuming that $\theta = 130^\circ$, γ has a value of 485 dynes/cm. Converting dynes per centimeter to psi yields $D = 181/P$ when the pore diameter in micrometers is inversely proportional to the mercury intrusion pressure in psi. In this procedure, the precise measurement of the mercury volume at any pressure, and hence a means of gauging the volumes intruded into the filter, is assessed dilatometrically, a method offering great accuracy.

Whatever its virtues, the method has serious shortcomings. Badenhop (21) concludes that mercury porosimetry is unsuited to the pore-size measurement of microporous membranes, and Williams (22) states that, in principle, fewer than 20% of the largest apertures (pores) need be breached by the intrusion of mercury to fill the membrane entirely. The chief objection to

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mercury porosimetry arises from the artificialities its manipulations bear to the filtrative process, an operation that usually involves aqueous flow through a filter under rather moderate pressures, the very essence of the flow-pore regimen. In any case, using this procedure, measurements can be made of the cumulative volume of mercury introduced into a filter at different pressure levels. From this, the percentages of the various pore sizes become available, and also the pore-size distribution curve.

Early work was taken to suggest that membrane filters had a pore-size distribution of ±0.02 μm about their mean pore-size rating. This narrow distribution had significance, as it was suggested that these filters would be expected to exhibit "absolute retentions," and this was further supported by the successful use of such membranes in filter sterilizations. However, examination of four commercially available 0.45 μm-rated membranes, each from a different manufacturer, by mercury porosimetry demonstrated that none of the tested filters had pore-size distributions as restrictive as ±0.02 μm. Therefore, it has been stated that the high reliability of their 0.2 μm-rated membranes for filtration sterilization applications is, therefore, derived from one or a combination of other physical and/or physiochemical considerations (23).

FILTER DESIGN AND CONSTRUCTION

Filter Styles

Disk or flat filters were the first filter configuration used in the pharmaceutical industry, mainly as 293 mm disks within multistack stainless steel holding devices. The assembly of such housing was/is difficult as one works with wetted flat filters and has to be extremely careful not to damage the filter membrane. Also wrinkles or bends during assembly might cause problems during the filtration process. Disk filters are cut from the casted membrane sheet and are available in a large variety of size, either builds into a disposable plastic housing or placed into a filter holder with diameters from 4 to 293 mm. The most common 47 and 50 mm are utilized as microbial (analytical) assessment filter and can have different colors or colored grids printed on the membrane. Such analytical filters commonly have a pore size of 0.45 μm and utilize adsorptive polymeric materials, for example, nylon or cellulose nitrate (21,24). The reason for the material choice is the requirement of adsorptive capture of the organisms. The pore size is chosen to be 0.45 μm to assure the nutrient, on which the membrane is placed, penetrates through to the membrane surface to feed the captured organisms.

Since disk filters are restricted within its EFA, pleated filter cartridge designs were developed to increase the filtration area without increasing the footprint of the filtration system or filter holder (Fig. 2).

The primary stimulus to develop pleated membrane cartridges was the need of an increase in the filter area sufficient to secure the engineering advantages of lower applied differential pressures and larger volume flows. Achieving this goal in the pleated filter cartridge form meant, moreover, that less floor space needed to be allocated for filter installations. To replace a common 10 in. filter cartridge and to achieve its same EFA, fifteen 293 mm disks would be needed. Therefore, the footprint of such system is by far larger than the need of a 10 in. filter housing. Moreover, every disk filter required O-ring sealing, therefore the assembly was time consuming and insecure.

The first pleated membrane materials were cellulose acetate, cellulose mixesters, polyamide, and polyvinylidene fluoride. Commonly, these membrane materials were surface treated to achieve pleatability, wetability, and stability of the membrane, which required large water flush volumes before the filter could be used. Nowadays available pleated filters are composed of cellulose acetates, Teflons, polyvinylidene fluoride, polysulfone, polyethersulfon, nylon, etc. The pleating arrangement, the back-and-forth folding of the flat membrane filter on itself, permits the presentation of a large filter surface area within a small volume. A pleated membrane cartridge of some 2.75 in. (70 mm) plus in diameter and 10 in. (254 mm) in length can contain from 5 to 8 ft^2 (0.5 0.8 m^2) of filter surface, depending on the membrane thickness, prefiltration layers, and construction detail. Pleated membrane cartridges are also offered in various lengths from 2 to 40 in. and EFA s from 0.015 to 36 m^2. This range of sizes and EFAs are

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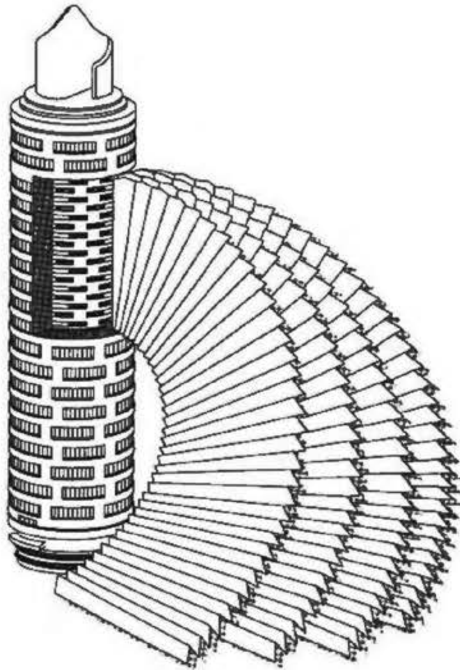

Figure 2 Typical pleated filter cartridge design. *Source*: Courtesy of Sartorius Stedim GmbH.

required for scale-up and down within the process and development steps. A pleated filter device should be able to scale-up linear from the preclinical volume size to process scale (11).

Typical construction components of the pleated filter cartridge are as follows:

The end caps are the terminals for the cartridge pleat pack and are responsible for holding the cartridge contents together. The end caps are also responsible for providing the seal between the cartridge and the O-ring recess on the cartridge-housing outlet plate or a base for the filter cartridge adapter, which can be of different shape and form. The adapter would be used to create a reliable seal between the filter and the filter housing. Polypropylene end caps are frequently adhered to the membrane pleat pack, by the use of a polypropylene melt softened preferably by fusion welding. In the past, stainless steel rings in the cartridge adapter stabilized the cartridge adapter against steam-induced dimensional changes and so preserved the integrity of the O-ring seal against bypass. The use of such dimension-stabilizing rings is made in the construction of pharmaceutical-grade cartridges intended for sterilization(s), especially when polypropylene end caps are involved. Nevertheless, it has been also found that such stainless steel ring, with different expansion rates during temperature changes, can also cause problems with respect to hairline cracks and fissures within the adapter polymer or the welding sites. This could go so far that the adapter damage does not allow proper O-ring sealing. This effect often has been seen with adapter, which has not been molded from one piece. The welding starts cracking, liquid penetrates into the stainless steel ring cavity, and expand during the next steaming (25). To avoid the differences in expansion of the support ring and the adapter polymer, most of the adapters are constructed with a polymer support ring.

The outer support cage is responsible for forming the outer cylinder of the cartridge and for holding the pleated internal contents together. The outer support cage also provides for a backpressure guard in preventing loss of filter medium integrity as a result of fluid flowing in the opposite direction under excessive backpressure. Additionally, it eases the handling of the filter cartridge during installation. The user does not come in direct contact with the pleats and damage can be avoided.

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The upstream nonwoven support layer serves as a multipurpose component. Pleating, and the assembly of the membrane into cartridge form, requires its inclusion in the cartridge. The supportive outer pleated layer aids in protecting the filter medium throughout the cartridge pleating and assembly operation. The material also serves as a prefilter to extend the useful service life of the final membrane that lies beneath it. Lastly, the support maintains the structure throughout fluid processing. Without this layer, the pleats under pressure might be compressed, limiting the filter area available to the fluid processing.

The drainage or downstream nonwoven support screen, similar to the upstream filter pleat support, stabilizes the pleating of the pleat pack. Moreover, it keeps the filter medium pleats separated during fluid processing to assure that maximum filtration area is open for optimum flow rates and drainage of remaining filtrate, that is, reducing the dead volume or otherwise trapped fluids. The filter arrangement of the microporous membrane sandwiched between the support and drainage layers, all simultaneously pleated, is often called "the filter pack" or the "pleat pack."

The filter cartridge inner core serves as the inner hollow tube on which the pleated pack is supported. It confers strength on the cartridge assembly. This component also determines the final assembly length of the cartridge. Lastly, the core is the outlet port of the cartridge. Through its perforations, the filtered fluid passes to be guided to the outlet plate of the filter housing. The cartridge core should not be flow limiting but can be in high-flow applications, that is, air filtration or water filtration with prefilter cartridges.

The filter membrane is the center of the filter cartridge, responsible for removal of the contaminants. Solutions permeate into and through the filter medium and into the cartridge inner core, then proceed through the adapter assembly and effluent piping. Once the filter medium has become fully wetted, processing can be continued until one of several flow decay indicators signals the need for cartridge replacement, as customer preference dictates.

Cartridge designs can be manifold and fit for the application. Not only size differences are applicable, but also cartridge adapters, that is, plug-ins, which fit into filter housings sockets and recesses. Single open-ended filter cartridges with bayonet locking are mainly used for sterilizing grade filter cartridges due to the reliability of the fit into the housing. Bypass situations have to be avoided, which can only be accomplished, if the sealing between the filter cartridge and its holder is snug. In the case of the string-wound cartridges, no end caps are used, because the avoidance of product bypass is not as critical as in sterilizing grade filtration (11); only the double open-end cartridges and the adapter pieces need be stocked.

In microporous membrane applications, frequent use is made of the single open-end 10 in. cartridge, usually in T-type housings. Therefore, such a unit is manufactured with an integral end cap. Such cartridges are also constructed in 20 and 30 in. lengths. Attempts have been made to offer pharmaceutical manufacturers the versatility of 10 in. single and double open-end units to be assembled via adapters with O-rings. Since such an arrangement increases the critical sealing area, its acceptance has been limited. The more widespread use in critical pharmaceutical manufacture is of single open-end 10, 20, and 30 in. cartridges.

The O-ring materials used are also of critical importance, as the chemical compatibility of the O-ring material has to be determined toward the fluid to be filtered. The O-ring is the critical area of the separation between up- and downstream side, therefore any incompatibility might be a hazard to the filtrate quality. Furthermore, in instances of multiple steam sterilization, the O-ring material has to be checked for so called heat set. The O-ring experiences the pressure points from the housing wall and the cartridge adapter. When the temperature is elevated, as in the steaming process, the O-ring starts deforming at the pressure points. If the O-ring material is not flexible enough, the deformation (heat set) will be maintained. The O-ring will commonly show an oval shape. It is important that O-rings are visually inspected on a routine basis to see whether the O-ring is deformed. Any heat set might result into a bypass situation. Ethylene propylene diene monomer (EPDM) O-ring materials showed so far the highest heat set tendency, nevertheless, are very compatible to chemicals. Silicone has commonly a high flexibility and low heat set (5).

The resulting increase in the EFA reflects two factors in addition to the cartridge diameter. The first consideration is the diameter of the center core of the cartridge. Each pleat consists of a membrane layer or of multiple membrane layers, sandwiched between two

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protective layers whose presence is necessary to avoid damage to the membrane in the pleating process, and which serve usefully in the finished cartridge as pleat separation and drainage layers. As a consequence of this sandwich construction, each pleat, naturally, has a certain thickness. Fewer of these thicknesses can be arranged around a center core of narrower diameter. Therefore, increasing the diameter of the center core increases the extent of its perimeter and the number of pleats that can surround it. This governs the number of pleats possible in the pleat pack that can comprise the membrane cartridge, thus increasing its EFA.

To define a cartridge, designations must be made of such considerations as its pore-size label (3), its diameter, its length, the type of outlet, for example, the O-ring(s) sizes, the configuration of the outer end, for example, open or closed, with or without fin, the type of O-ring or gasket seal, for example, silicone rubber, EPDM rubber, and any nonstandard features. Manufacturer product numbers serve as shorthand substitutes for the detailed specifications.

The optimum number of pleats to be arranged about a center core of a filter cartridge may reflect the filtrative function for which it is intended (25,26). In the handling of rather clean, prefiltered liquids, as in most pharmaceutical final filtrations, relatively few particles require removal. A crowding of as large a number of pleats as possible to enhance the filter area may be acceptable because the pleat separation layers will operate to make even the crowded surfaces individually available to the liquid being filtered. Where there are high solids loadings in the liquid, or a viscous fluid, a different situation may result however. The particles being removed may be large enough to bridge across a pleat to block the interval between two adjacent pleat peaks. Or, being small, they may, after their individual deposition on the filter, secrete and grow large enough to cause bridging. Whatever the mechanism, the bridging serves to deny the liquid, being processed, access to useful flow channels bordered by membrane.

In practice, pleated cartridges are built for general usage in what is still an artful construction (5,25,27). Nevertheless, there is said to be available an empirically developed formula that relates the outer cartridge diameter to the maximum core diameter, and to the number of pleats of given height that should be used.

Care must be taken to protect the surface of the membrane during the pleating operation and to avoid damage to the filter structure. Both these objectives are furthered by sandwiching the membrane between two support layers and feeding the combination to the pleater. The outlying support layers protect the membrane surfaces. Nevertheless, the fleeces have to be chosen properly; for example, a fleece too coarse could press too much on the membrane at the pleating curvation and starts pressing into the membrane. In Figure 3, one can see the result of

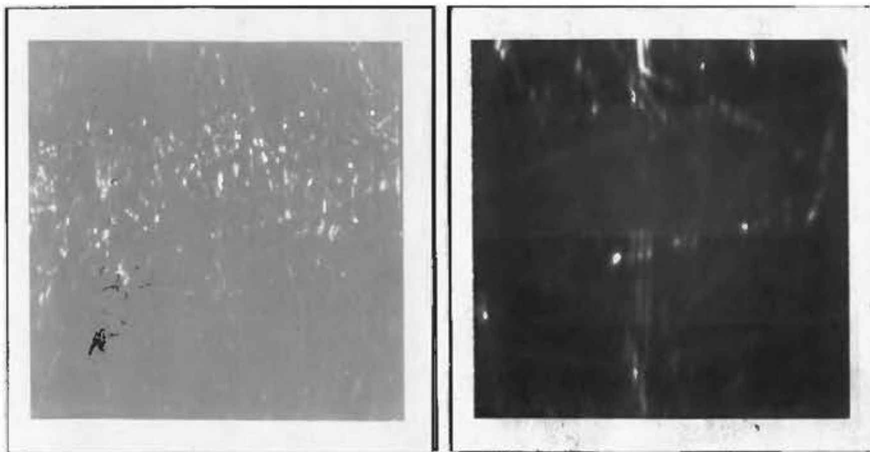

Figure 3 Prefilter impressions on a PTFE membrane filter membrane. *Source*: Courtesy Sartorius Stedim GmbH.

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coarse fleece compression on a PTFE membrane, which weakens the membrane and might be detrimental in long-term use of the filter. Air filters are used over a long period and experience multiple in-line steam sterilization. If the membrane shows impressions by the coarse filter fleece, this commonly means that the filter membrane in this area is thinning. Multiple steam sterilization could exaggerate this thinning and flaws can develop. On the other hand a fleece, which is too soft will not support the membrane sufficiently. Usually soft fleeces have a high-fiber density and a small-fiber diameter, which means liquid, would be bound within the fiber structure. Such phenomenon needs to be avoided, for example, in air filtration, because it could cause water logging.

Additionally, the sandwich in its thickness minimizes opportunities for the membrane to be too strongly compressed at the pleat. What is required is a pleat having some radius of curvature rather than a sharp, acute angle of fold. This prevents the membrane from being subjected, at the pleat line, to forces in excess of its mechanical properties as expressed in the magnitude of its tensile and elongation values. Different polymeric materials will, of course, have different tensile and elongation qualities; various materials differ in their brittleness. Additionally, sharp pleat edges or pleatings with a high pleat density will have a gap in between the pleats, which would result into capillary activity; that is, in air filtration condensate could potentially be trapped in between the pleats and the air filter might experience water blockage. Therefore, filter designs and construction require thorough investigation in development to achieve the best performance ratios. In instances the highest effective filtration are in the confined construction of a filter cartridge might not be the optimal solution, as the pleat density becomes too high. Nevertheless, EFA should also not be too low as it will influence the flow rate and total throughput. Decreasing the diameter of the center core will serve to lessen the number of pleats, although in applications that require a high flow; for example, air, the inner core becomes the flow restrictor. Therefore, the inner core again needs to be optimized to the filter cartridge utilization. For example, a 28-mm core diameter will require a 40% to 50% higher differential pressure than a 35-mm inner core to achieve an air flow rate of 100 scbm. This differential pressure increase might not seem to be high, but the costs involved running such pressure difference is substantial.

The disk and cartridge filters of commerce are usually disposables. It is their housings and holders, usually of metal, that are permanent. However, filters encapsulated into plastic housings have been devised wherein the entire unit is disposable (Fig. 4). There are advantages to these devices. Among them is that many are available in presterilized conditions, by gamma irradiation, steam, or ethylene oxide. Another advantage, therefore, is their ready availability. That they are disposables does not necessarily militate against the economics of their usage.

Figure 4 Different filter capsule devices. *Source*: Courtesy of Sartorius Stedim GmbH.

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Calculations show that where labor costs are reckoned, the installation of a single 293-mm filter disk in its housing is more costly than the equivalent filtration area in the form of a disposable filter device. The use of the disposables entails very little setup time and no cleanup time. There is no need to sterilize the already presterilized units. Disposal after the single usage eliminates risks of cross-contamination. Pleated disposable device show commonly better performance due to the prefilter fleeces and sometimes prefilter membrane in front of the final filter membrane. Therefore, 293 mm disk filters could potentially also be replaced by 150 or 300 cm^2 disposable devices, even when such have a smaller EFA.

The venting of disposable filter devices has been the subject of good design considerations. One disposable-capsule manufacturer has positioned the vents at the highest point of the containing shell, exactly where they are most effective. Another design utilizes a self-venting device in the form of a hydrophobic membrane. This permits the self-venting of air while safeguarding against the passage of liquid or contaminants (in either direction).

There are often ancillary advantages to the use of disposable filter devices. Some manufacturers construct their shells of transparent polymers so that the filtration process is observable. The instruments are compact and relatively lightweight, hence, easy to handle; also their construction does not lack the sophistication of their metal housing contained counterparts. Many of the disposable units are equipped with vent plugs and drain plugs. The identifying description they bear on their outer casings, make their traceability, in accordance with FDA record requirements rather certain (3). Product and batch numbers become part of the permanent operational record. Above all, the use of these disposables obviates the need to expense or amortize stainless steel filter holders. No capital expenditures are involved.

The use of disposable filters can reduce costs in respect of cleaning, which would occur with stainless steel filter housings after every use. Cleaning validation, which needs to be performed with fixed equipment like filter housings, will be greatly reduced. The disposable filters do not go through such cleaning regime and therefore the validation of cleaning exercises is avoided. For this reason and the convenience of the use of disposable filters, the biopharmaceutical industry has switched more and more to capsule filters instead of filter housings. Commonly, a disposable capsule filter is connected to a disposable bag, both are available in different sizes for the individual purpose. Once the capsule filter is connected to the bag the entire setup is gamma irradiated to sterilize. Certainly the filter material and polymers need to be gamma stabile otherwise particle shedding or an excess amount of extractable can occur.

Another advantage of disposable filter capsules is the fact that the user will not encounter the product filtered. This certainly could be the case when using cartridge filters within a housing. The cartridge has to be removed from the housing at the end of the filtration run, that is, the user probably comes in contact with the filtered product remaining on the filter cartridge and housing, which may need to be avoided due to health hazards or biological activity. Disposable filters create the opportunity to replace a filter without being in contact with the product.

The disposable filter devices are available in a variety of constructions, whether disk, multidisk, pleated cylinders of various lengths and of different EFA s. Their expanse of filter surface runs from 4 mm disks suitable for affixing to hypodermic needles to 30 in. capsules of about 180 ft^2 (1.8 m^2). The filters are made of a variety of polymeric filter materials, both hydrophilic and hydrophobic, namely, cellulose esters, polyvinylidene fluoride, polysulfone polyethersulfone (26), nylon, polyethylene, Teflon, etc. Their shells can be composed variously of polycarbonate, polyethylene, but most often polypropylene.

The use of most cartridge filters accords with FDA emphasis on record keeping. Despite all the care with which filter manufacturers pack flat disk filters, the membranes themselves are unlabeled. Cartridge filters are, however, available with identifying data (28). Most are identified with some code, if not on the cartridge itself then on its container. Some manufacturers stamp the cartridge end cap with the part number, its pore-size identity, and its lot number as well. Indeed, some manufacturers even number each cartridge consecutively within each lot. Should the need ever arise to trace the components and history of these filters, and of their components, the ability to do so exists. Batch records in concert with the appropriate manufacturing QC records make this possible.

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Because of the fragility of most membrane filters, appropriate and even extreme care is to be used in their handling. In the case of cartridge filters, this practice continues. However, the actual membrane surface of these instruments is out of reach ordinary handling. There is, therefore, far less possibility of damage to the filters. Overall, cartridges are used mostly for the more rapid flow rates and/or the large-volume filtration productions they enable, a consequence of their aggrandized EFAs.

Membrane Configurations

A homogenous membrane is usually a dense film ranging from 10 to 200 μm thickness. A porous membrane understandably has a porous structure (29 31,70). The size and shapes of the pores largely determine the separation characteristics. As the pore size increases, the separation become more similar to that of a filter, where compounds are allowed to pass on the basis of size. The intrinsic nature of the material can still have an effect on the separation by, for instance, slowing the passage of one compound due to molecular attractions. The pores in a porous membrane can be cylindrical. However, it is more common that the pores have a range of size and are tortuous path. The separation mechanism for porous membranes is more similar to conventional filtration larger particles or compounds cannot pass through the pores and are therefore retained.

In addition to porous and homogenous, membranes can be classified as symmetric and asymmetric. Symmetric membranes have a structure that is consistent throughout. Homogenous membranes are symmetric. Porous membranes can also be symmetric with pore sizes and pore shapes consistent throughout. Nevertheless, there is no general understanding, defined parameters, or equation to classify a membrane as asymmetric or symmetric. Therefore, each membrane manufacturer and user have their own approach to the definition of this membrane parameter.

In general, an asymmetric membrane has a structure that is different on the surface compared to the interior. In one case, the surface, or skin, may be dense and the rest of the membrane is porous (Fig. 5). Or the surface may have different-sized pores compared to the membrane interior. Since most of the separation characteristics result from the surface, the surface can be tailored according to the application. For instance, a porous membrane could have an integral dense skin on the surface. The dense skin is much thinner (0.1 1.5 μm) than a comparable homogenous membrane and therefore has higher permeability. This sort of membrane is usually more effective for gas separations and for reverse osmosis than a dense homogenous membrane made of the same material. The porous substructure of the membrane gives the membrane strength without adding resistance to mass transfer.

The process to create a dense membrane skin on a porous support from a single material is difficult. It is often simpler to deposit a coating on a porous membrane surface that acts as the dense, highly selective membrane. The coating can be a different polymer that is more

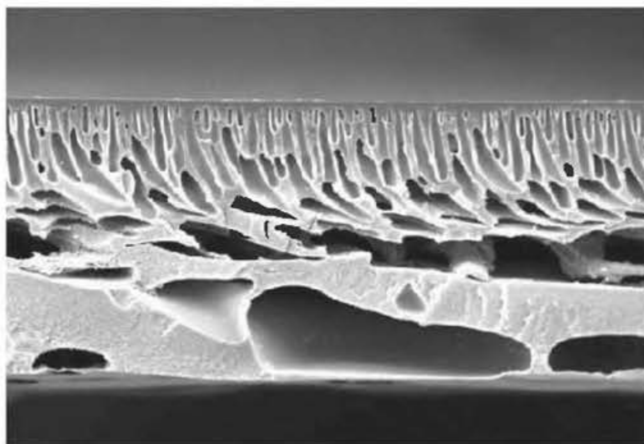

Figure 5 Skin layer membrane. *Source*: Courtesy of Sartorius Stedim GmbH.

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selective for the application than the intrinsic properties of the polymer support layer. The polymer can be applied by many techniques, the most important of which are dip coating and interfacial polymerization.

SEPARATION MECHANISMS

Sieve Retention

Sieve retention of particle capture is the one most evident in common filtration experiences. It occurs whenever a particle is too large to pass through a filter pore. It is a geometric or spatial restraint. This type of particle arrest is considered "absolute" (but only for the defined size of the particle) in that it is independent of the filtration conditions. The applied differential pressure does not influence it, unless the level is so high as to deform either the particle or the filter pore, an occurrence not alleged in pharmaceutical filtrations. Sieve retention is also free of the influences of the particle challenge level. Regardless of the number of particles confronting the filter, if each is too large to pass the filter pores then none will be able to do so, and all the particles, regardless of number, will be retained. Additionally, the particle retention will be independent of the suspending liquid vehicle as defined by its ionic strength, pH, surface tension, temperature, viscosity, and presence or absence of surfactant, etc. (17,71).

Adsorptive Retention

Zsigmondy (32) pointed out that the filter surface has a certain adsorbing capacity whose affinity must first be satisfied before unhindered passage of the dispersed phase through the filter may occur. Numerous investigators have since noted specific adsorptions of many entities. Elford (29) reported that dyes could adsorptively be removed from true solutions by collodion membranes (cellulose nitrate, one of the most adsorptive materials). The strong adsorption tendencies of the cellulose nitrate polymer had also been noted by Elford (13) in the case of viruses. The use of membrane filters adsorptively to collect and isolate nucleic acids, enzymes, single-strand DNA, ribosomes, and proteinaceous materials in scintillation counting operations is well established. Moreover, such adsorptive retentivity is utilized nowadays in chromatography and membrane adsorber steps of the downstream purification process. Bovine serum albumin, antigen/antibody, and antibody complex, and specific binding and receptor protein adsorption to cellulose nitrate has been shown to occur. Berg et al. (33) investigated the adsorption of both inorganic and organic compounds on polymers such as cellulosic filter papers, nylon, polyethylene, and cellulose diacetate dialysis membranes. That water-soluble organics could adsorptively be removed from aqueous solutions by filters was observed by Chiou and Smith (34). These investigators were thus led into a rather thorough study of such adsorptions by filters. Undani (35) and Brose et al. (36) studied the adsorptive sequestration of such preservatives as benzalkonium chloride, chlorocresol, and chlorhexidine acetate from their solutions by membrane filters. The adsorptive removal of flu vaccine impurities and antibodies onto membrane filters has been reported (37,38). Inorganic particulate matter can be removed filtratively through the adsorption mechanism. It is thus well documented that molecules and materials can be adsorbed onto filters to become filtratively removed thereby.

There are several references in the literature pertaining to the retention of organisms by contact with filter surfaces. Pertsovskaya and Zvyagintsev (39) report that films of such polymeric as polyamide, polyacrylate, polyethylene, and cellulose triacetate adsorb different groups of different bacteria. Zierdt (40) and Tanny et al. (41) demonstrated that bacterial adsorption could take place on the surfaces of membrane filters whose pores are many times larger than the organisms. During the laboratory development of a lyses-fractionation blood culture technique, Zierdt et al. (42) at the National Institutes of Health noted that both gram-negative and gram-positive organisms were attracted to the membrane materials during filtrations. The filters were composed of polycarbonate and cellulose mixesters. Furthermore, the arrested organisms resisted removal by the mechanical or adsorptive action of backwashing with buffer. These investigators were therefore enabled to use filter membranes with porosities much larger than would normally be expected to arrest the bacteria whose retention they wished. The organisms involved were *Escherichia coli* and *Staphylococcus aureus*.

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Figure 6 Microorganisms captured on glass fiber. *Source*: Courtesy of Sartorius Stedim GmbH.

Sterility was neither sought nor obtained. Beyond doubt, however, the bacterial capture by the membrane filters involved adsorptive arrest.

Zierdt et al. (42) found that a higher percentage of bacterial retention occurs at low organism concentrations, about 500 to 100 CFU/mL. At higher levels of 10^8 to 10^9 CFU/mL, increasing percentages of *E. coli* pass through the membranes, although a larger total number is retained. These phenomena accord with adsorption. Retention was investigated as a function of the filter pore-size ratings. As expected, the larger the pore-size ratings of the filters, the greater the amount of bacterial passage. At low bacterial numbers, 6.2×10^2 CFU for *E. coli* and 7.3×10^2 CFU for *S. aureus*, apparently no *E. coli* pass a 3.0-μm filter nor *S. aureus* a 5.0-μm filter. All of the above reflect the influence that organism concentration exerts on filter capture efficiency during adsorptive sequestrations.

It also has been shown that *B. diminuta* (formerly *P.*) can be retained by adsorptive glass fiber filters (Fig. 6). It is evident that many of the organisms are retained by contact capture rather than by sieve arrest; the filter pores, the spaces among the fibers, obviously are often too large to serve as retaining orifices.

Surface phenomena, such as adsorptions, can be related to forces between molecules, especially to an asymmetry or unbalance of forces at an interface. The hydrogen bond is an example of an asymmetric force caused by the presence of unequally shared electrons within the water molecule. This creates partial charges, electrical in nature, on atoms of the water molecules. The oxygen atom, retaining more than its share of electrons, becomes negatively charged. The hydrogen atoms of the water molecule, possessing a smaller portion of electrons, become positively charged. Opposite charges attract one another; similar charges repel one another. The opposite electrical signs on separate water molecules result in adsorptive interactions called the hydrogen bond. The electrical forces between ions are full charges. The electrons composing them are completely donated by one atom of a molecule and are fully received by another atom. They are not partial charges. The attractive forces resulting from partial charges are short range and electrostatic, and are usually characterized as van der Waals forces, such as govern the condensation of a vapor into a liquid.

Energy is required to effect the separation of a bacterium from a surface to which it is adsorbed. The energy level is an expression of the bonding strength, the adsorption, between the organism and the polymer surface. This, in turn, depends on the contributions made to the bond by the membrane surface and by the organism. It is not surprising, therefore, that

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different filter surfaces bond differently with a given organism, and that different organisms adsorb differently to a given filter surface. Additionally, product parameters, that is, the filtrate properties do influence the adsorptive capture or attractiveness of and to a surface tremendously.

Ridgway (43) found that mycobacterial adhesions to polyamide type reverse osmosis membranes showed a 5- to 10-fold greater affinity than did their adsorptions to cellulose ester RO membranes. It may be speculated on the basis of this finding that strong bacterial adsorptions to polyamide (nylon) membranes account for the sterilizing effects of such 0.2 μm-rated membranes, even when they are more open than their counterparts not composed of this polymer. Ridgway also found that different organisms had different propensities to adsorb to surfaces, as gauged by biofilm formation. It is possible, however, that this adsorptive phenomenon reflected particular morphological features of the different organisms rather than their molecular makeup.

An interesting example of adsorptive interconnections formed between molecules of entirely different compositions is given by the actions of surfactant molecules. These compounds reduce the high interfacial surface tensions that separate nonpolar hydrocarbons, such as oils, from polar liquids such as water. Emulsifications in particular characterize the forces at play; for example in liquid-liquid contacts. As stated, the interfacial tensions are highest between those of different polarity and structure. High interfacial tensions are a negative for interactions among different molecular structures. Reduced interfacial tensions favor such interconnections. Wetting agents or surface acting agents perform their functions by reducing the interfacial tensions. Consider the immiscibility of oil and water, one a hydrophobic, nonpolar compound and the other a molecular structure so polar as to be importantly significant in hydrogen bonding interactions. Tobolsky (44) points out that the molecular structure of sodium oleate, a surface tension reducer for water, has a "strongly polar head and a long nonpolar tail." The nonpolar molecular group of the surfactant attaches to a nonpolar oil molecule. Its polar group attaches to the polar water molecules. The sodium oleate molecule in so doing bridges the polarity difference between the oil and water. The result is an aqueous emulsification of the oil; the one wetting the surface of the other. The bonding versatility of the surfactant molecules bridges the differences in polarity between polar and nonpolar compounds. An oil in water emulsion results.

In specific applications the adsorptive sequestration mechanism is sought. Its application to pharmaceutical filtrations will certainly require in-depth validation. If adsorptive sequestration is a major function of the retentivity of a filter, such retentive effectiveness needs to be analyzed utilizing process conditions and the actual product to be filtered. Under no circumstances can filtrative efficiency be assumed, if not documented by bacteria challenge test results. This also is valid for claims of endotoxins removal by filtration. Such removal requires qualification over the filtration period at very defined process conditions. Any changes in the process conditions can alter the filtration result. For this reason, any sterilizing grade filter needs to be validated using the product as the challenge test carrier and the actual process conditions.

FILTRATION CONSIDERATIONS

Pressure/Flow

The differential pressure is the prime motivator of a liquid's flow and determinant of its rates. Most of the aqueous preparations dealt within the pharmaceutical industry are Newtonian fluids. By definition, a direct and linear relationship exists between their clean (absent particles) flow rates and the differential pressure (Δp) for a given EFA. To overcome the resistance to flow caused by placing a filter in the path of a clean fluid stream, a higher differential pressure or a more extensive EFA is required. The difference in the pressures, upstream and down, determines the rate of flow. Alternatively, an increase in the available filter area will compensate for the decrease in the flow rate. Maintaining a constant flow while changing one of these parameters by some percentage or multiple necessitates a balancing of its influence by compelling an opposite change in the second parameter by the same percentage or multiple.

The flow rate of clean liquids, those not encumbered with particles, is also affected by viscosity and by temperature, its reciprocal, as also by its degree of adsorptive interactions, if

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any, with the filter. The latter may result in a plasticization of the polymeric matrix. This may manifest itself by a swelling of the matrix into its open spaces, the pores, which, in response, may be accompanied by their shrinkage. This would impede the liquid flow (45).

The initial rate of flow of "real fluids," defined as those containing particles, will progressively undergo reductions proportional to the pore blocking occasioned by the filter's ongoing particle retentions. Moreover, decreases in flow rates may result due to the differential pressure's compaction of filter cakes that may form on the filter's surface. This contaminated rate of flow reflects the rate of flow decay. The total throughput, an important goal of the filtration as measured by weight or volume, will depend on the sizes and shapes and numbers of the total suspended solids' components (TSS), and on the particle size/pore size relationships governing particle retentions and pore blockage. Throughput is flow rate dependent over time. Its utilitarian value is self-evident in terms of yield.

The filter system's design, whether of the membrane combinations or of other structural features, may vary the resistance to flow. For example, a single membrane layer will have a higher flow rate than the same filter area for each of a double-layer combination.

On the basis of the extrapolation of graphs from filter makers catalogues, it may appear that a high-flow-rate system (e.g., 400 L/min) can be designed with one or two 10 in. (25.4 cm) cartridges. This design, however, would not take into account the resistance to flow of the filter housings. Filter manufacturers publish graphs of flow rates that are fairly linear within a range. Within this range, most of the differential pressure is used to drive the fluid through the filter and only a small portion is involved in overcoming the flow resistance of the housing. Filter manufacturers generally supply data concerning the rates of flow through their various filters (and housings) as a function of incremental pressure differential, for example, 4 L/min/psi for a 0.2 μm-rated membrane. One must choose as a flow limit that differential pressure that will not cause the flow capabilities of the filter housing to be exceeded.

Viscosity/Temperature Effects

Flow rate is the easiest to measure from among the filter properties of interest: flow rate, throughput, and extent of particle removal. Flow is, for most fluids, a product directly defined by the differential pressure and is inversely moderated by viscosity. Viscosity, in turn, is reciprocal to temperature. Rates of flow can be varied by manipulating the differential pressure and the temperature/viscosity relationship.

Flow rate information is normally given for water. Since the rate of flow varies inversely with viscosity, the flow rates for more viscous liquid media will be reduced proportionately and must be corrected for. Water, the standard, has an assigned numerical viscosity value of 1 centipoise (cP). A liquid having a viscosity of 3 cP will flow one-third as fast; a liquid whose viscosity is 36 cP will flow 1/36th as rapidly, etc. The viscosity effect on rates of flow is not exact, as it ignores liquid/filter interactions that in their extreme manifest themselves in filter swelling and other expressions of incompatibility. Fortunately, substituting other liquids for water generally minimizes these aberrations.

Viscosity may also affect particle retentions. Higher viscosities exert greater "drag" forces on a suspended particle. The "drag" is the partial charge attractive force manifest between particle and the liquid molecules that are themselves interconnected by hydrogen bonding. It is hypothesized that the effect on a particle could be tantamount to a higher delta pressure in that its "drag" may exert a force sufficient to frustrate its adsorption onto a filter's surface or even be strong enough to cause the particle's desorption.

Generally, liquids tend to be less viscous at elevated temperatures and filter more rapidly. The heating of liquids to effect more rapid filtration is usually not used however. In particular, the thermal denaturation of protein poses a threat, and certain ranges of temperature over time are encouraging to organism growths. In studying liquid behavior, note should be made of the liquid's temperature.

Contamination Load

There are threats posed by contaminants to drug preparations. None is more significant than the presence of organisms. They are the contaminants whose presence, if tolerable, is least desirable. To make certain that the filters employed for their removal are sufficiently efficient for the task,

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high standards are set by the governmental authorities for confronting the filters with large quantities of live organisms. From tests performed on the filter effluents, microbiological assaying can determine the extent of completeness with which they were filtratively removed. In instances where sterility is the goal, the complete retention of the organisms must be ascertained. In processing operations, it is necessary to use methods, techniques, all equipment, and appurtenances, etc., that have been validated to attain that accomplishment.

The FDA sets the standard microbial challenge at 1×10^7 CFU/cm^2 of EFA. The thinking is that if a filter can perform against so large an amount of organisms, it can surely manage the removal of a lesser number. As logical as this standard may seem, it does not meet with universal agreement. The EMEA requires that the final filter in the filter train should not be confronted with more than 10 CFU/100 mL organism level (46). Larger numbers than that will require a "sterilizing grade" filter downstream of it. Apparently, the thinking is that the fewer the number of organisms that confront a filter, the less likely is one to penetrate it.

Complicating the situation is the myriad number of different organisms that are extant. Dealing with this complication is made easier by designating a particular microbe to serve as a model for those most likely to be encountered in pharmaceutical settings. For this purpose, *B. diminuta* ATCC 19146 is used. It usually serves adequately, but with exceptions. *B. diminuta* is of a size that suits it to be sieve retained by 0.2 μm-rated membrane filters. There are, however, organisms that undergo changes in their size on exposure to certain liquids. Other organisms are known to be alive, but whose existence cannot be verified because they are not amenable to cultivation.

Compatibility

The filter must be compatible with the liquid it is to filter. It should not undergo chemical attack, nor should its pore structure become modified lest its retention capabilities become altered. Chemical degradation is usually obvious in the filter's physical property changes it causes. Color changes in the filter or its embrittlement may signal oxidative free radical attacks (Fig. 7). Hydrolytic actions by strong acids or bases may partially destroy the filter or permit it to swell in water. Solvents will either gradually dissolve the filter or cause it to soften noticeably. In rarer instances, it will show distortive shrinkage or develop scalloped edges or wrinkles. Gross incompatibilities are not difficult to detect. More subtle effects are of greater concern because they may be overlooked.

Since the chief filter action is the removal of particles from their suspensions, changes in their pore structures are to be guarded against. Their occurrence can be detected by comparing filter's bubble point values before and after its exposure to the liquid for at least the time

Figure 7 Oxidative coloration and disinte gration of an air filter. *Source*: Courtesy of Sartorius Stedim GmbH.

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period from their initial contact through to the filtration's completion. Determining their diffusive airflow rates before and after contact will be an even more sensitive gauge. The bubble point test will disclose enlargements of the largest pores, if any. The diffusive airflow will reveal alterations in pores of any size. While the former test will be more pertinent to the implications of particle passage, the latter, being more inclusive of all size pores, may by its sensitivity indicate a potential for unwanted pore-size mutations.

Incompatibilities that may alter pore shapes or sizes or otherwise compromise microbial retentions are of prime importance. A lack of compatibility can also serve to weaken the mechanical strengths of a filter by a plasticizing action making it less able to withstand its former differential pressure. This most likely will also reduce the filter's density. A larger or faster leaching of compounds from within the polymeric filter may result. The quantity of extractables and the speed of their transfer from within the filter body will also be expedited.

INTEGRITY TESTING

Sterilizing grade membrane filters are required to be tested to assure the filters are integral and fulfill the purpose. Such filter tests are called integrity test and may be performed before, but must be performed after the filtration process. Sterilizing grade filtration would not be admitted to a process, if the filter would not be integrity tested in the course of the process. This fact is also established in several guidelines, recommending the use of integrity testing, pre- and post filtration. This is not only valid for liquid, but also air filters.

Integrity tests, such as the diffusive flow, pressure hold, bubble point, or water intrusion test, are nondestructive tests, which are correlated to the destructive bacteria challenge test with 10^7 CFU/cm^2 *B. diminuta* (47 49). Derived from these challenge tests specific integrity test limits are established, which are described and documented within the filter manufacturers literature. The limits are water based, that is, the integrity test correlations are performed using water as a wetting medium. If a different wetting fluid, or filter, or membrane configuration is used, the integrity test limits may vary. Integrity test measurements depend on the surface area of the filter, the polymer of the membrane, the wetting fluid, the pore size of the membrane, and the gas used to perform the test. Wetting fluids may have different surface tensions, which can depress or elevate the bubble point pressure. The use of different test gases may elevate the diffusive gas flow. Therefore, appropriate filter validation has to be established to determine the appropriate integrity test limits for the individual process.

Bubble Point

Microporous membranes pores, when wetted out properly, fill the pores with wetting fluids by imbibing that fluid in accordance with the laws of capillary rise. The retained fluid can be forced from the filter pores by air pressure applied from the upstream side to the degree that the capillary action of that particular pore is overcome (Fig. 8). During the bubble point test, the pressure is increased gradually in small increments. At a certain pressure level, liquid will be forced first from the set of largest pores, in keeping with the inverse relationship of the applied air pressure P and the diameter of the pore, d, described in the bubble point equation:

$$P = \frac{4\gamma \cos \theta}{d}$$

where γ is the surface tension of the fluid; θ, the wetting angle; P, the upstream pressure at which the largest pore will be freed of liquid; d, the diameter of the largest pore.

When the wetting fluid is expelled from the largest pore, a bulk gas flow will be evaluated on the downstream side of the filter system during a manual test. The bubble point measurement determines (to a certain degree) the pore size of the filter membrane, that is, the larger the pore the lower the bubble point pressure. Therefore, filter manufacturers specify the bubble point limits as the minimum allowable bubble point and correlate the bubble point test procedure to the bacteria challenge test. During an integrity test, the bubble point test has to exceed the set minimum bubble point for it to pass.

Key for a successful bubble point test is the qualified wetting fluid and its surface tension. The bubble point will be highly influenced by surface tension changes within the

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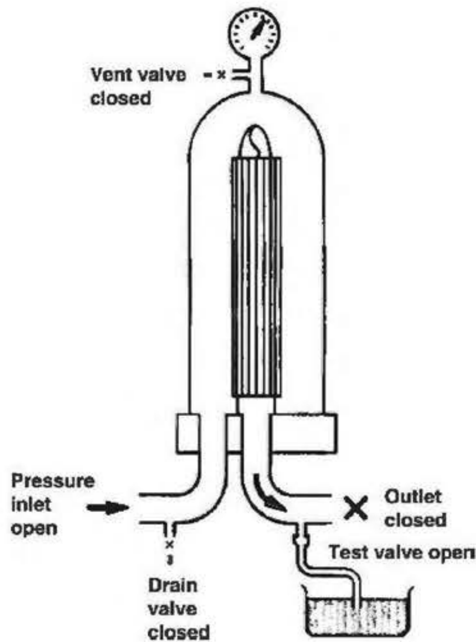

Figure 8 Manual bubble point test setup. *Source*: Courtesy of Sartorius Stedim GmbH.

Table 2 Bubble Point Values for Different Wetting Agents Using Cellulose Acetate 0.2 μm

Product	Bubble point value (bar)
Water	3.20
Mineral oil	1.24
White petrolatum	1.45
Vitamin B complex in oil	2.48
Procainamide HCl	2.76
Oxytetracyline in PEG base	1.72
Vitamin in aqueous vehicle	2.07
Vitamin in aqueous vehicle	2.69

Source: Courtesy of Sartorius Stedim GmbH.

wetting fluid. Table 2 shows different possible wetting fluids and the bubble point changes of such, utilizing the same membrane.

Yet, the surface tension of the wetting liquid, as also its viscosity, diminishes with rising temperature, while the angle of wetting increases, and its cosine decreases with the hydrophobicity of the filter polymer. The less hydrophilic the polymer, the less perfectly does it wet, particularly with aqueous liquids. Therefore, the bubble point is a specific product of the each particular filter/liquid couple. It varies from one polymer to the other and therefore bubble point values given and obtained are not equal, even for the same pore-size rating. That the bubble point of a filter differs for different wetting liquids is commonly known. That it differs also for polymeric materials is less appreciated.

The bubble point test can only be used up to a certain filter size. The larger the filter surface, the larger the influence of the diffusive flow through the membrane. The diffusive flow would cover the actual bubble point due to the extensive air flow. Therefore, the bubble point finds its ideal use with very small system to medium size systems (some mention the critical borderline to use the bubble point is a 3 × 20 in. filter housing, depending on the pore size).

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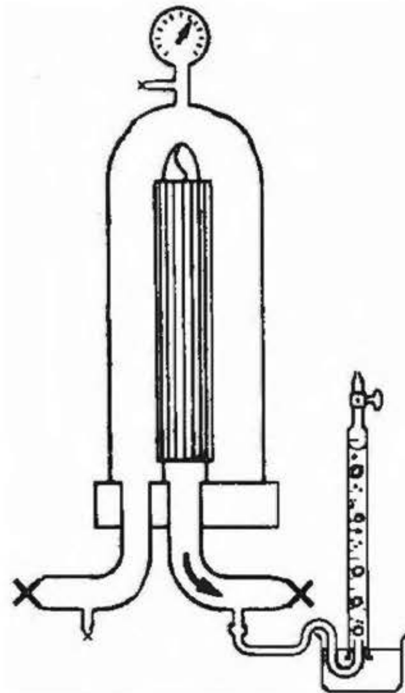

Figure 9 Manual diffusive flow test setup.

Diffusive Flow

A completely wetted filter membrane provides a liquid layer across which, when a differential pressure is applied, the diffusive airflow occurs in accordance with Fick's law of diffusion (Fig. 9). This pressure is called test pressure and commonly specified at 80% of the bubble point pressure. In an experimental elucidation of the factors involved in the process, Reti simplified the integrated form of Fick's law to read

$$N = \frac{DH(p_1 \quad p_2)\rho}{L}$$

where N is the permeation rate (moles of gas per unit time); D, the diffusivity of the gas in the liquid; H, the solubility coefficient of the gas; L, the thickness of liquid in the membrane (equal to the membrane thickness if the membrane pores are completely filled with liquid); $P\ (p_1 \quad p_2)$ is the differential pressure; and ρ, the void volume of the membrane, its membrane porosity, commonly around 80%.

The size of pores does only enter indirectly into the equation; in their combination they comprise L, the thickness of the liquid layer, the membrane being some 80% porous. The critical measurement is the thickness of the liquid layer. Therefore, a flaw or an oversized pore would be measured by the thinning of the liquid layer due to the elevated test pressure on the upstream side. The pore or defect may not be large enough that the bubble point comes into effect, but the test pressure thins the liquid layer enough to result into an elevated gas flow. Therefore, filter manufacturer specify the diffusive flow integrity test limits as maximum allowable diffusion value. The larger the flaw or a combination of flaw, the higher the diffusive flow.

The diffusive flow cannot be used for small filter surface, due to the low diffusive flow with such surfaces. The test time would be far too extensive, and the measured test value too unreliable to be utilized. Nevertheless, the diffusive flow as well as the pressure drop test are best used for larger filtration surfaces, where the bubble point test finds its limitations (50).

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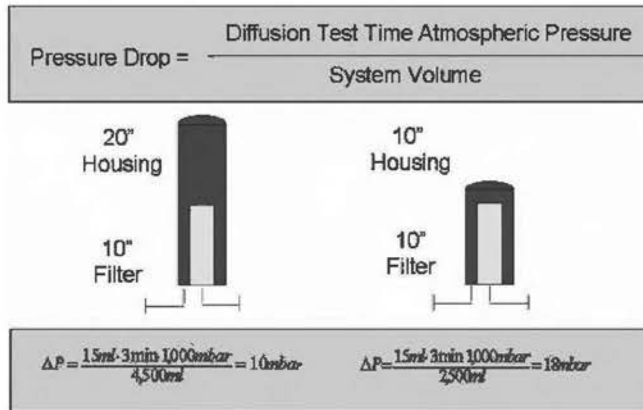

Figure 10 Influence of the upstream volume to the pressure decay measurement.

Pressure Hold

The pressure hold test is a variant of the diffusive airflow test. The test setup is arranged as in the diffusion test except that when the stipulated applied pressure is reached, the pressure source is valved off. The decay of pressure within the holder is then observed as a function of time by using a precision pressure gauge or pressure transducer.

The decrease in pressure can come from two sources: (*i*) the diffusive loss across the wetted filter. Since the upstream side pressure in the holder is constant, it decreases progressively all the while diffusion takes place through the wetted membrane and (*ii*) source of pressure decay could be a leak of the filter system setup.

An important influence on the measurement of the pressure hold test is the upstream air volume within the filter system. This volume has to be determined first to specify the maximum allowable pressure drop value. The larger the upstream volume, the lower the pressure drop (Fig. 10). The smaller the upstream volume, the larger the pressure drop. This means an increase in sensitivity of the test, but also an increase of temperature influences, if changes occur. Filter manufacturers specify maximum allowable pressure drop values, utilizing their maximum allowable and correlated diffusive flow value and convert this diffusive flow maximum with the upstream volume into a maximum allowable pressure drop.

Another major influence on pressure decay is temperature. Any temperature change during the test will distort the true result, as an increase in the temperature will lower the pressure drop and a decrease will artificially elevate the pressure drop. Therefore, the temperature conditions during the test should only vary slightly. This also means that the wetting agents used should have a similar temperature as the environmental temperature surrounding the test setup. Temperature differences between the wetting solution and the test gas and the temperature of the environment will influence the true test result. The pressure hold test is an upstream test, even when performed manually.

Multipoint Diffusion Test

In single-point diffusive flow testing, the test is performed at a defined test pressure, which is commonly around 80% of the bubble point value. Therefore, the area between the diffusive flow test pressure and the bubble point value is not tested and stays undefined. In comparison, the multipoint diffusive airflow test is performed at a multitude of test pressures. Usually, this test is performed with an automated test machine, which allows defining the individual test pressure points with high-test accuracy. Moreover, once the pressure points are defined the machine performs the test without the need of supervision. Therefore, valuable time and resources are not bound. To the benefit of data storage, the test machines also print an exact graph of the test performed, therefore any irregularities will be detected.

Multipoint diffusion testing has advantages over single-point diffusive testing, because it can more rapidly detect a pending product failure due to gradual filter degradation (51,52). A

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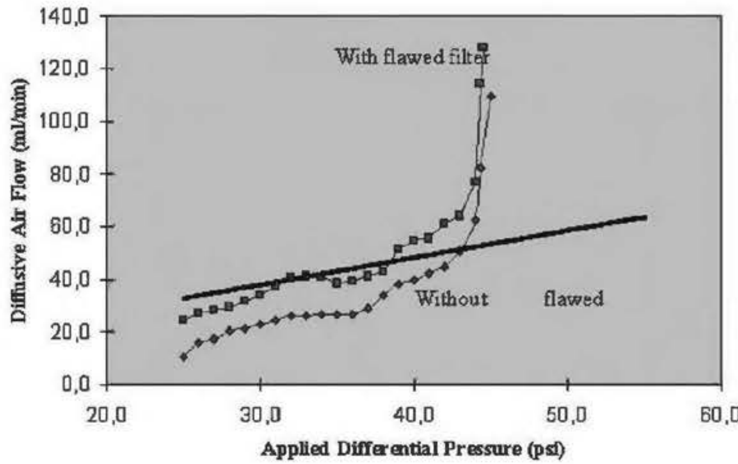

Figure 11 Multipoint diffusive flow testing to detect a flawed filter in a multiround filter housing.

multipoint integrity test could indicate a trend of increasing diffusion over time that might be overlooked with single-point diffusion testing and even through bubble point testing. Furthermore, the multipoint diffusion test seem to have the ability to test multiround housings reliably (Fig. 11). As described in the bubble point and diffusive flow test section, both tests have their limitations accurately integrity testing multiround filter housings. A single-point diffusive flow test may not be able to find a flawed filter within the multitude of filters. The bubble point may be covered by an excessive diffusive flow.

In any case, the multipoint diffusive flow test seems to be able to find a flawed filter due to the change of the slope of the linear section of the diffusive flow. A single flawed filter cartridge can be detected within a three round filter housing, where a single-point test would not have determined the defect. Such test may take longer in its test time, but will add to the overall accuracy of integrity testing multiround housings.

In instances, the multipoint diffusion test finds also its usefulness in the analysis of failed filter integrity tests. For instance, when a filter failed the single-point diffusive flow test or bubble point test, one should aim for testing the filter with a multipoint diffusion test to see the entire graphic. This result could be compared to the graphs established during the performance qualification phase. Commonly, there are distinct test graphics, which show whether the filter has a flaw and if so what the cause of the flaw could be.

Water Intrusion Test

The water intrusion (also known as water pressure hold) test is used for hydrophobic vent and air membrane filters only (53 55,73). The upstream side of the hydrophobic filter cartridge housing is flooded with water. The water will not flow through the hydrophobic membrane. A specified gas pressure is then applied to the upstream side of the filter housing above the water level. This is done by way of an automatic integrity tester. A period of pressure stabilization takes place over a specified timeframe, recommended by the filter manufacturer, during which the cartridge pleats adjust their positions under imposed pressures. After the pressure drop stabilizes, the test time starts and any further pressure drop in the upstream pressurized gas volume, measured by the automatic tester, signifies a beginning of water intrusion. The automated integrity tester is sensitive enough to detect the pressure drop. This measured pressure drop is converted into a measured intrusion value, which is compared to a set intrusion limit, which has been correlated to the bacteria challenge test. As with the diffusive flow test, filter manufacturers specify a maximum allowable water intrusion value. Above this value a hydrophobic membrane filter is classified as nonintegral.

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FILTER VALIDATION

The probably most thorough guidance (recommending) document is the PDA Technical Report No. 26. It describes filter structures, usage, purpose, and integrity testing. Most important is the description of the filter validation needs within the actual filtration process (2,3,11,56,72). The document defines the needs for viability, product bacteria challenge, extractable, particulate, and adsorption testing. Before the PDA Technical Report has been accomplished, FDA's Guideline on Sterile Drug Products Produced by Aseptic Processing has been the guidance document of choice. The 1987 guidance has been replaced by a new guidance document of September 2004, which adopted multiple descriptions of Technical Report #26. Similarly, the ISO 13408 leans very much toward Technical Report 26 and describes appropriate filter validation very much in the fashion of the mentioned report.

The United States Pharmacopeia (USP) as well as any other pharmacopeia should be closely monitored, due to the descriptions of required limits for particulate, endotoxins, and biocompatibility testing. Within the filter manufacturers filter qualification tests, pharmacopeial limits are analyzed and need to be met by the filter products distributed. These tests commonly cover toxicological, endotoxins, extractable and particulate tests, which are well defined with the , and any filter utilized within the biopharmaceutical industry requires being compliant. These tests are the basic requirements to be fulfilled and should not be misinterpreted as appropriate filter validation studies. Filter validation requires to be performed with the actual drug product to be filtered under process conditions. Most of the pharmacopeial tests are performed with water or other pure solvents.

A guideline of considerable importance, especially in regard to revalidation or second filter vendor implementation, is the FDA Guidance for the Industry Changes to an Approved NDA or ANDA, section VII, Manufacturing Process (1999). This guideline describes distinctively the different needs of prior approvals, if changes have been made to the actual processes. It defines what is a minor, moderate, or major change with respect to filtration devices and changes to sterilizing grade filters and what are the consequences.

A guideline that causes confusion and insecurities with respect to redundant 0.2 µm filtration is the EMEA CPMP/QWP/486/95 Guideline (46). This guidance document defines a maximum allowable bioburden level of 10 CFU/100 mL in front of a 0.2-µm sterilizing grade filter. If this level is exceeded, a bioburden reducing filter has to be used in front of the sterilizing grade filter. Although, the guidance leaves room for interpretation in respect to what type of filter this could be, it also states that the use of a second 0.2 µm in front of the final 0.2 µm filter does not required additional validation. It is now debatable whether the bioburden limit defined is reasonable, as well as the excessive reliance on pore size.

Bacteria Challenge Test

Before performing a product bacteria challenge test, it has to be assured that the liquid product does not have any detrimental, bactericidal, or bacteriostatic effects on the challenge organisms, commonly *B. diminuta*. This is done utilizing viability tests. The organism is inoculated into the product to be filtered at a certain bioburden level. At specified times, defined by the actual filtration process, the log value of this bioburden is tested. If the bioburden is reduced due to the fluid properties different bacteria challenge test modes become applicable. There are three bacteria challenge methodologies described within the PDA Technical Report No. 26; high organisms challenge, placebo (modified product) challenge, and product recirculation with a challenge after recirculation. If the mortality rate is low, the challenge test will be performed with a higher bioburden, bearing in mind that the challenge level has to reach $10^7/cm^2$ at the end of the processing time. If the mortality rate is too high, common definition is more than 1 log during processing time, the toxic substance is either removed or product properties, for example, pH, temperature, etc., are modified. This challenge fluid is called a placebo. The third methodology would be to circulate the fluid product through the filter at the specific process parameters as long as the actual processing time would be. Afterward the filter is flushed extensively with water and the challenge test, as described in ASTM F838-05 (57) performed. Nevertheless such challenge test procedure would be more or less a filter compatibility test.

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Sterilizing grade filters are determined by the bacteria challenge tests. This test is performed under strict parameters using a defined solution (57). In any case, FDA nowadays also requires evidence that the sterilizing grade filter will create a sterile filtration, based on the actual process parameters, fluid properties, or bioburden found. This means that bacteria challenge tests have to be performed with the actual drug product, bioburden, if different or known to be smaller than *B. diminuta* and the process parameters. The reason for the requirement of a product bacteria challenge test is threefold. First of all the influence of the product and process parameters to the microorganism has to be tested. There may be cases of either shrinkage of organisms due to a higher osmolarity of the product or prolonged processing times or starvation due to the extreme low organic properties of the fluid. Secondly, the filter's compatibility with the product and the process parameters has to be tested. The filter should not show any sign of degradation due to the product filtered. Additional assurance is required that the filter used will withstand the process parameters, for example, pressure pulses, if they occur, without influencing the filter's performance. Thirdly, there are two separation mechanisms involved in liquid filtration: sieve retention and retention by adsorptive sequestration. In sieve retention, the smallest particle or organism size is retained by the biggest pore within the membrane structure. The contaminant will be retained, irrespective of the process parameters. This is the ideal situation. Retention by adsorptive sequestration depends on the filtration conditions. Contaminants smaller than the actual pore size penetrate such and may be captured by adsorptive attachment to the pore wall. This effect is enhanced using highly adsorptive filter materials, for example, glass fiber as a prefilter or polyamide as a membrane. Nevertheless, certain liquid properties can minimize the adsorptive effect, which could mean penetration of organisms. Whether the fluid has such properties, which will lower the effect of adsorptive sequestration, and may eventually cause penetration has to be evaluated in specific product bacteria challenge tests.

Extractable/Leachable Test

Besides the product bacteria challenge test, tests of extractable or leachables substances have to be performed. Previous reliance on nonvolatile residue (NVR) testing as a method of investigating extractable levels have been dismissed by the regulators in 1994 (58). Since then extractable/leachables analysis from filters and other components are routinely done by appropriate separation and detection methodologies. Extractable measurements and the resulting data are available from filter manufacturers for their individual filters.

These tests are performed with a specific solvent, for example, ethanol and water at "worst case" conditions. Such conditions do not represent true process realities Therefore, depending on the process conditions and the solvents used, explicit extractable tests have to be performed. Formerly, these tests were done only with the solvent used in the drug product formulation, but not with the drug ingredients themselves, because the drug product usually covers any extractable during measurement. Nevertheless, recent findings have been presented, which reported the possibility to evaluate extractable utilizing the actual drug product as the extraction medium. Such tests are conducted by the validation services of the filter manufacturers using sophisticated separation and detection methodologies such as GC-MS, FTIR, RP-HPLC, UV-VIS, GPC-RI, HPCE, and SFC (59,60). These methodologies are required due to the fact that the individual components possibly released from the filter have to be identified and quantified. Elaborate studies on sterilizing grade filters, performed by filter manufacturers, showed that there is neither a release of high quantities of extractable (the range is ppb to max. ppm per 10 in. element) nor have toxic substances been found.

Authorities and organizations nowadays seem to have changed their focus to other equipment used within the industry, for example, disposable media bags, plastic vials, tubing, or stoppers. Prefilters also have become a target. There are already extractable studies performed on a variety of pleated prefilter types of polypropylene and glass fiber. Nevertheless, lenticular and string wound prefilters, widely used within the biopharmaceutical industry still, have to undergo such investigation.

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Chemical Compatibility Test

The PDA Technical Report No. 26 describes very specifically "A simple chemical compatibility chart will often not provide enough information for predicting filter system compatibility, thereby requiring additional testing." Chemical compatibility has been underestimated in the past and reliance has been focused on chemical chart of pure solutions. The aim of chemical compatibility testing is to find subtle incompatibilities, which may happen due to a mix of chemical components and entities or specific process conditions. Elevated temperatures or prolonged filtration times may result in a filter incompatibility, which has to be investigated.

Even though the filter membrane is not compromised in respect to its retentivity, it can add extractable/leachables. Therefore, appropriate compatibility tests have to be performed with the actual drug product at the process conditions. Commonly, integrity tests before and after the submersion of the filter in the product will show whether an incompatibility exists. Sole reliance, though, should not be on integrity testing. NVR testing parallel to integrity testing may be the procedure of choice, in case the filter is integral but shows elevated extractable levels. Scanning electron microscopy may be utilized to see any chemical attacks on the membrane surface. Above-mentioned bacteria challenge tests and extractable analysis also contribute valuable information with respect to the filters compatibility.

Other Requirements

Particulates are critical in sterile filtration, specifically injectables. The USP (and BP (British Pharmacopeia) quote specific limits of particulate level contaminations for defined particle sizes. These limits have to be met and therefore the particulate release, if any, from sterilizing grade filters has to meet these requirements. Filters are routinely tested, evaluating the filtrate with laser particle counters. Such tests are also performed with the actual product under process conditions to prove that the product and especially process conditions do not result in an increased level of particulates within the filtrate. Specific flushing protocol, if necessary, can be established for the filters used. These tests are also useful for any prefilter as it reduces the possibility of a particulate contamination within the process.

Additionally with certain products loss of yield or product ingredients due to adsorption shall be determined. Specific filter membranes can adsorb, for example, preservatives, like benzalkonium chloride or chlorhexidine. Such membranes need to be saturated by the preservative to avoid preservative loss within the actual product. This preservative loss, for example, in contact lenses solutions, can be detrimental due to long-term use of such solutions. Similarly problematic would be the adsorption of required proteins within a biological solution. To optimize the yield of such proteins within an application, adsorption trials have to be performed to find the optimal membrane material and filter construction, but also flow conditions and prerinsing procedures. Any yield losses by unspecific adsorption can cost millions due to lost product and its market value. Adsorption studies are helpful to optimize downstream process in regard to any yield loss that in turn can influence production capacity.

To summarize, most of the described validation effort have to be performed and are part of the validation master file of a particular process and drug product. Interestingly enough, validation receives emphasis and attention, but one should also never forget training. Without appropriate personnel training any validation effort done is in vain. Filter users should also test their staff to be able to handle filtration, the sterilization, and integrity test of such installation and sanitization. Training has to be the focus of all operations to deliver a reliable and sustainable process.

Product Wet Integrity Test

More often, postfiltration integrity testing is performed by using the product filtered as the wetting agent, due to the fact that a flush with water may need a copious amount of such. Certainly, the contact between certain membranes and various pharmaceutical preparations can produce depressed bubble points compared with the values for water (Table 2). The depressed bubble point can be restored, more or less, but mostly less, by abundant washing of the filter with water, depending on the filter material and/or product ingredients used. Some subtle wetting effects, adsorption or fouling involving product ingredients, may be at work

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here whose surface physics is not comprehended. In addition, the surface tension differences between the product and water are contributory to the anomaly.

Often, efforts are made to flush the filter with water before running the final integrity test so that pre- and postfiltration bubble point tests using water are obtained for comparison. However, even copious water flushing may not restore the water bubble point. For example, it was reported that nylon membranes became fouled by proteins in an albumin filtration process that resulted in filters not being wetted with water leading to false-negative results. Same was found with products containing Tween. Even after large water flush volumes, the surface tension reducing properties were seen. In such cases, pre- and postfiltration comparisons may usefully be performed using product as wetting agent for the filters. The displacements in bubble values being ascribed to unknown wetting effects, but largely to the influences of the surface tension values of the product, are assumed not to reflect on the organism removal capabilities of the membrane.

However, regulatory authorities also advocate to perform bacteria challenge tests with the actual product under process conditions. Such challenge tests, involving also viability testing, confirm the filter's retentivity; moreover they reveal any negative influences of the product toward the challenge organism (17).

Parker (61) determined the acceptable minimum bubble point for a given type of filter using product as wetting medium in accordance with the formula

$$P_p = \frac{P_o \cdot P_m}{P_w}$$

where P_p is the minimum acceptable product bubble point; P_o, the observed bubble point using product; P_w, the average of the water bubble points observed for samples of the filters (commonly 3 filters from 3 different batches); and P_m, the filter manufacturer's stated minimum allowable bubble point. Enough filters or filter devices are secured from each lot of the subject filter type to yield an acceptable average value. Testing is performed for each product being filtered using 47 mm disk filters or small-scale pleated filter devices.

Desaulnier and Fey (62) confirmed Parker's findings. Parker and Desaulnier and Fey describe the exact protocols by means of which the product bubble point may be determined. The latter authors also describe an apparatus suitable for the purpose.

Usually, the evaluation of the so-called product integrity test values requires three filter membranes or devices of three different lots, that is, nine tests in total. At one point, it was recommended that one of these filter lots must be close to the minimum allowable water bubble point value given by the filter manufacturer to ensure retentive capability at the established limit values. This factor is now included within the corrected product-wetted bubble point value evaluation.

Commonly the diffusive flow is measured at around 80% of the bubble point pressure as the test pressure. A drug product, which is used to wet the filter membrane, can shift the bubble point value and therefore one has to determine the test pressure to be used to perform a product-wetted diffusive flow test. This determination of the test pressure is commonly done by a series of product and water-wetted bubble point tests. The values of these tests will then be used to calculate the product-wetted test pressure for the diffusive flow test. The PDA Technical Report 26, 1998, describes the formula in detail as other have done it before (61 63).

$$\mathrm{TP_{PW}} = \mathrm{MTP_{WW}} \frac{\mathrm{PBPavg}}{\mathrm{WBPavg}}$$

where $\mathrm{TP_{PW}}$ is the product-wetted test pressure; $\mathrm{MTP_{WW}}$, the water-wetted test pressure specified by the filter manufacturer; PBPavg, the average product-wetted bubble point; and WBPavg, the average water-wetted bubble point both evaluated during the test series described earlier.

Once the product-wetted test pressure is evaluated, then the product-wetted diffusive flow limit will be determined. For this determination one will first water wet the membrane filters and perform repeated test of minimum of three different filter lots. After this is done, the filters should be dried or rinsed sufficiently with the product. Again the filters will

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undergo several, previously defined diffusive flow tests. The values of all tests can then be used to calculate the maximum allowable diffusion limit for a product-wetted filter using the formula, described by the PDA Technical Report 26, 1998:

$$DFL_{PW} = DFL_{WW}\frac{DF_{PW}}{DF_{WW}}$$

DFL_{PW} is the maximum allowable product-wetted diffusive flow limit; DFL_{WW}, the water-wetted diffusive flow limit defined by the filter manufacturers correlation; DF_{PW}, the product-wetted diffusive flow, and DF_{WW}, the water-wetted measured diffusive flow value. This test restricts itself to the single-point diffusion test, and one can argue about its accuracy (50). In any case, the more accurate test would be a multipoint diffusion test, evaluating the slope of the diffusive flow at the test pressure with different wetting media. Such test purely from a statistical point of view is more accurate besides plotting the entire diffusion graph. The plot of the graph will not only show the slope of the linear section of the diffusive flow, but also a shift of the exponential, bubble point, sections. Further details can be found in the multipoint diffusion test paragraph.

APPLICATIONS

Liquids

An ideal liquid filter would have following attributes:

- The filter should have a high flow rate at low differential pressures
- The filter should have a high total throughput performance
- The filter must retain contaminants, especially microbial, as defined and desired
- The filter membrane polymer should be low adsorptive, if used in specific applications, which do not have the need of adsorptive retention
- The filter requires to have a high mechanical robustness to withstand possible differential pressure surges
- The filter requires to withstand up to 134°C steam sterilization temperatures or be able to be gamma irradiated

Such filter represents the "perfect world." Most commonly one has to settle for a compromise between the listed attributes. There is not such filter that is optimal for every application. Liquid filters are commonly developed and designed to work best within specific applications.

Solvent (API) Filtration

Filters within this type of application require being highly compatible to aggressive solutions or process parameters. The fluids are highly aggressive and the best filters to be found for such applications are polyamide or polytetrafluoroethylene membrane polymers. Before the filters can be used within such application, appropriate performance qualification trials should be completed to assure the filter is compatible with the fluid and process parameters. Especially subtle incompatibilities can cause major problems if not determined early enough. The liquid filters used in these applications are polishing or bioburden reducing filters. The bioburden in aggressive solutions may mainly be spores as the fluids are commonly bactericidal. However, any potential contaminant requires to be removed to avoid microbial contamination in the downstream process.

Ophthalmics Filtration

Ophthalmic solutions require two main attributes, besides the obvious microbial retentivity: (*i*) high total throughputs for cellulosic-based complex solutions with high viscosities and (*ii*) low unspecific adsorption for solutions containing preservatives like benzalkonium chloride or chlorhexidine. The total throughput determination can happen via 47 mm disk trials followed by verification trials with small-scale pleated devices. The solutions are complex and may require prefilter/final filter combinations. During the filterability trial work

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it is of importance to sample the filtrate in specific volume or time intervals and check the filtrate in regard to the preservative concentration, if applicable. Preservative adsorption to the membrane filter polymer is not uncommon and requires to be established to avoid an out-of-specification event in terms of the preservative level within the final container. Low adsorptive polymeric membranes should be used, for example, polyvinylidene fluoride, cellulose acetate, or modified polyethersulfone. It might be that the solution requires to be recirculated over the membrane to saturate the adsorptive sites, before the solution is redirected to the fill line. Often ophthalmic solutions are filled utilizing blow-fill-seal equipment, which could mean a prolonged filling period. If this is the case, the filter validation (retention study) requires including such prolonged filling period.

Cell Culture Media

Media are available in a large variety from different raw material sources and of different compositions. Moreover, the raw material quality experiences seasonal, dietary, growth, and regional variations, which makes it in instances difficult to define the exact performance of a raw material. This factor can be challenging when filtration systems have to be determined and sized. Therefore, the main performance criterion for filtration systems for media is total throughput or filter capacity, the total amount of fluid that can be filtered through a specified filtration area. Filters used in media filtration should be optimized to achieve the highest total throughput and will be tested accordingly. To achieve reliable data, it is always of advantage when the test batch is at the lower end of the quality specification to gain a worse case scenario. Temperature, differential pressure, and pretreatment of the filter play an important role in performance enhancement of the filter system (64,65). For example, it has been experienced that lower temperature of the media filtered and even the filter system might enhance the total throughput by 30%. The flow rate will be affected by the higher viscosity, but again the essential performance part is not flow but total throughput. Too high flow rates in the filtration of biological solutions showed the negative side effect of gel formation on the membrane and therefore premature blockage. To start with lower differential pressure has been seen advantageous, as again gel formation and/or cake compaction will be avoided. The lower the differential pressure at start of the filtration, the better the performance. A preflush of the filter system with preferably cold buffer will also enhance the total throughput. Hitting the filter with just the media has been found to foul the filter faster and therefore reduce the filter's capacity. In instances it is necessary to utilize prefilter combinations to avoid fouling or blocking of the sterilizing grade or 0.1 μm final filter element. These combinations need to be determined in filterability trials to gain the most optimal combination to filter the particular media and to size the system appropriately.

Another important, but often overlooked factor of media filtration is the influence of unspecific adsorption of the filter material. To separate lipids in the media raw material adsorptive filter media are desired. However, in cell culture media, especially containing growth promoters, unspecific adsorption has to be avoided. Certain membrane polymers do have a higher unspecific adsorption. Sometimes, the membrane polymer can be of similar type, but the surface treatment of the polymer is different or the design of the filter device is different. In any case, high unspecific adsorption can have an influence on growth promoters like IGF.

Buffer Filtration

Since buffers are commonly of high purity the filter performance criteria focuses on flow rate and not total throughput. A premature blocking of the filter is often not experienced. Flow though is the determining factor of process time within the buffer preparation process. The faster the flow rate of the filter the higher the equipment utilization. The better the flow rate of the filter the lower the required EFA, respectively the cost per liter will be reduced. For example, a low flow rate (2500 L/hr), 0.2 μm-rated filter would require 48 minutes to filter a 2000-L volume versus only 20 minutes for a high-flow filter (6000 L/hr). This would reduce equipment's used time by half or the EFA could be reduced, which would cut filter costs.

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Another important factor to consider is the buffer's pH range or the variety of buffers used. One can find certain pharmaceutical processes where the pH ranges from 1 to 14, which in some polymers are capable to withstand and others not. Again filter vendors are aware about this fact and developed high-flow filters most often with a polyethersulfone base polymer as this material is compatible over the entire pH range.

Gases

An ideal gas filter requires listed attributes:

- The filter must retain microorganisms and other contaminants, even under unfavorable conditions such as high humidity
- The filter must have high thermal and mechanical resistance
- The filter ought to withstand multiple steam sterilization cycles
- The filter should allow high gas flow rates at low differential pressures
- The membrane should be hydrophobic to resist blockage by elevated humidity, condensate, or water remaining from a water intrusion test
- The filter must not release fibers
- The filter must be integrity testable with a test correlated to removal efficiency with various contaminants.

An optimized air filter can be described as a perfected recipe, as all components utilized, the design of the filter fulfilling the listed attributes. If only one of the attributes is focused on, it might be that the filter is imbalanced and does not meet other criteria of importance.

Fermentor Inlet Air

Air volume requirements vary during the different stages of fermentation and therefore the filter system used in large volume fermentation are of different sizes. For example, filter systems size used for seed fermentors are usually single 10 or 20 in. filter cartridges, whereby filter systems for large-scale fermentation may utilize a multifilter housing of 96 round 30 in. cartridge elements, depending on the product and fermentor volume. Such filter systems are used on a long-term basis and could be used for over a year; that is, these kind of filters require a high mechanical and thermal stability. These filters withstand sterilizing cycles of up to 200 cycles at temperatures up to 134°C. The filter manufacturers optimize membrane filter cartridges to create high flow rates at very low differential pressures. Membrane materials are chosen to achieve high pore volumes, hydrophobicities, and sterile filtration capability. Construction of the filter cartridges is optimized to avoid water logging and high velocities and the resulting pressure losses.

Fermentation can last up to 1 to 20 days therefore high security is required. It would be disastrous in terms of the product intake costs and running costs, if such large-scale fermentor became infected after several days of fermentation.

Fermentor Off Gas

Off-gas filtration becomes a major concern and requirement, especially in the biotech industry. In the past, most of the fermentation sites did not use any exhaust filter system, because the head pressure in the fermentor eliminated the risk of contamination from the off-gas side. Because of new restrictions and an environmental awareness, more and more facilities employ exhaust filter systems. The aim here is not to protect the fermentor content, but rather the environment to microbial contamination. For this reason different separation methods were evaluated, for example, cyclones in combination with depth filter types or heat. Both methods do not create the assurance level needed, beside one is very costly, therefore the use of membrane filter system becomes common practice.

The filtration of exhaust gases creates some major problems due to the moisture content that the gas carries. The gas is usually warm and saturated with moisture due to the contact with the fermentation medium. When the exhaust gas cools down, large amount of condensate will be the result, which could water block the sterilizing grade filter and increase the pressure

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drop over the filter. An increase in pressure drop means an immediate rise of the head pressure of the fermentor, which needs to be avoided. Particles and microbial contamination carried over from the fermentor into the exhaust stream could block the filter device. The retentive ability of such filter needs to be high, otherwise organisms will penetrate through the filter element. In some instances the microbial load of such filter can be up to 10^{11} organisms in a seven-day fermentation (66). Often enough, when the fermentor runs at the highest rate, foaming of the fermentor broth happens and can blind the filter.

Heating by steam and electrical tracing of the filter housings or pipework will avoid condensation due to the fact that the system temperature is held above the dew point of the air. If condensation occurs, the filter needs to be able to achieve required flow rates due its hydrophobicity. Condensate will be repelled and drained from the system. To assure that the filter will not loose its performance due to foam reaching the membrane either antifoam agents or mechanical foam breakers like demisters and baffles or cyclones can be used. Antifoam agents can have the disadvantage of fouling downstream processing filter devices rapidly, besides the antifoam agent needs to be sterile filtered. Mechanical foam breakers and cyclones (67) avoid the mentioned disadvantages, but usually work only effectively at specific air flow rates that vary from phase to phase of the fermentation process. Fine aerosol carried over from demisters or cyclones can be separated by tight depth filter cartridges containing polypropylene fleeces. These filters are very sufficiently protecting the costly sterilizing grade filter, due to the high dirt load capacity and a certain hydrophobicity, which avoids blocking of the depth filter fleeces. The void volume of these, filter is very high, therefore the pressure losses are minimal. Particles and microbial contamination will be greatly reduced and the lifetime of the sterilizing grade filter prolonged.

Vent Filters on Tanks

Every pharmaceutical application uses tanks, containers, and/or bags for a wide variety of purposes, for example, storage tanks for intermediate or final products, water storage tanks, transport vessels, or mixing tanks. Some applications only require a depth filter type, due to the product or medium stored in the tanks, which is unsuitable for any microorganism growth. Nevertheless, most of the tank-venting applications have in common that the air supplied into these tanks needs to be sterile and free of contaminations, usually achieved via a sterilizing grade, hydrophobic membrane filter.

When liquid is drawn from the tank or added to the tank, the air needs to be vented into or from the tank. Open to the atmosphere, the air needs to filtered through a sterilizing grade vent filter to avoid any contaminations, which could spoil the product stored in the tank. Often, the product fed into the tank is sterile filtered and the tank steam sterilized, therefore the vent filter needs to perform with highest security to ensure sterility. The filter needs to be and remain hydrophobic to avoid any condensate blockage and microbial growth on or within the filter matrix, especially when the vent filter is used over a long period of time without steam sterilization. This is the case on water storage tanks, which hold water of lower quality than Water for Injection (WFI), which is stored at around 80°C. The water temperature of WFI avoids or restricts microbial growth, but has the side effect of a high condensate rate, due to the high humidity of the air overlaying the hot water. A condensation of water on the filter cartridge can be avoided by using heat-jacketed filter housings, preferably an electrical heater. When using such heat-jacketed housing the filter cartridge must be visually checked on a routine basis, some manufacturers quote around every three months, to see whether parts of the filter are damaged by oxidization.

Nonvacuum resistant tanks, which are steam sterilized, need to be equipped with an appropriately sized vent filter system to overcome the condensation vacuum, created by the collapsing steam when the tank cools down (64,68). If the filter system is not correctly sized or the vent filter blocks due to a low hydrophobicity, the created vacuum could cause an implosion of the tank. Therefore, sizing of such vent filter systems is done by experienced and trained professionals.

The volume of some tanks is too vast to use a static vent filter system, at that point compressed air is pushed via a sterilizing filter into the tank to break the vacuum in the tank.

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Implosion of the tank can also be avoided by using burst disks or pressure relief valves, which open up when the vacuum in the tank reaches the allowable limit. Unfiltered air rushes into the tank and breaks the increasing vacuum, which means burst disks and pressure relief valves are just precautions in case of an insufficient working vent filter.

Vent filters on tanks and vessels are generally steamed from the reverse flow direction. In this instance, the differential pressure over the filter device during steaming needs to be operated carefully. Most of the filter manufacturers allow a maximum differential of 0.2 to 0.5 bar at around 134°C steam temperature. Steaming in reverse direction is usually more stressful to the filter construction. It is therefore advisable to integrity test the filter system after steam sterilization.

Autoclave and Lyophilizer Vent Filter

In the past, the vent filters used for autoclaves and lyophilizers were depth filter type cartridges, sometimes even coalescing type filters. Because of stringent quality standards and demands of the regulatory agencies, these filter were replaced by sterilizing grade membrane filters. When breaking the vacuum created in these machines, the air vented into the chambers can come in direct contact with the product. Therefore, it is of great importance that these filters stand up to the requirements set.

Main demand is the sterile filtration ability of the filters, which is achieved by several different sterilizing grade, 0.2 µm rated, membrane filters, available in the market. These filters are usually correlated to challenge tests, like the ASTM Bacteria Challenge test (57) or aerosol challenge tests, performed by the individual filter manufacturer or independent institutes. Having the ability to create a sterile filtrate does not mean that the individual filter will be the right choice for this type of application. Another important aspect is the hydrophobicity of the filter membrane and the construction of the cartridge, as pointed out in the section on sterilizing grade filters. If the hydrophobicity of the membrane material used is of lower value, the pore structure could be blocked by condensate, which is possible after steam sterilization. At this point, the vacuum in the chamber cannot be broken and the filter needs to be bypassed, which means the chamber is unsterile and the process will have to be repeated. It goes without saying that hydrophobicity is of major importance, yet in the field some filter users still utilize with filters of lower hydrophobicity. Some users were even advised to use hydrophilic sterilizing grade filters to overcome the use of wetting media like solvent/water mixtures, so they can use water to integrity test the hydrophilic filters. To create airflow through this type of filter the bubble point needs to be exceeded, even when heat-jacketed housings are in use. This not only creates insecurities, but process failures. The construction of the filter cartridge needs to be optimized so that condensate can run into the condensate chamber and drain. The size of the filter system used on these units is usually bigger, due to the amount of condensation and the low differential pressures, down to 10 mbar, especially close to the end of the venting process.

These filters must withstand a high amount of steaming cycles. Some large volume hospital sterilizers are used up to five times a day and more; that is, the filter will be steam sterilized five times. Certainly, these filters are not changed every time. The number of steaming cycles can be as high as 250 cycles. Often enough the steaming happens to be in reverse direction of the filter cartridge, which is a higher stress factor to the material and construction of the filter cartridge. The maximum differential pressure over the filter must be checked carefully, otherwise the filter could be damaged. Filter manufacturers quote maximum allowable differential pressure at elevated temperature of 134°C from 0.2 to 0.5 bar. As one recognizes there is a higher-risk factor of damage of these filter cartridges due to mentioned stress factors, and therefore these filters should be integrity tested on a routine basis.

In the past, the filters were either not tested and discarded after a certain period of time or tested off-line before steam sterilization. These days, filter manufacturers offer integrity test methods, which are able to integrity test the filter in-place, even after steam sterilization. These tests methods either accommodate the common solvent/water mixture to integrity test the filter via diffusion or bubble point test or just water for the water intrusion test. Moreover, manufacturers of autoclaves and lyophilizers have either incorporated fully automatic

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integrity tests methods in their equipment or advised their clients to install additional test equipment subsequently.

Filtration of Service Gases

Service gases, usually air and nitrogen, are used for pneumatic actuated valves and switches, head pressures of tank, transfer gases, drying purposes, and filling machines. These gases need to be sterile, because they are commonly supplied into clean room or sterile areas and come in contact with the product or the container, like vials, flasks, bottles, and tanks. Unfortunately, often enough these filters are overlooked, because there are so many in a standard pharmaceutical facility and sometimes not easily accessible or not obvious. This usually means that these filters are not integrity tested on a routine basis or not exchanged for a long period of time. Because of the more stringent requirements of the regulatory bodies, the awareness level for those filter units has increased and maintenance and quality assurance departments enforce checks on a regular basis.

With some exceptions, service gas filters are either not easily accessible or in pipe work, which is not steam sterilized. One major exception is blow-fill-seal filling machines. These filling machines mold the required containers, sterile fill them, and seal the containers. The need of an excessive amount of sterile air for extrusion, cooling, and overlaying purposes is obvious. Often these filling machines are equipped with up to four different air filtration units, for their different functions. Important here is that the air comes into direct contact with the plastic container and is introduced into the filling area (69). Therefore, the emphasis of routine steam sterilization and integrity testing of the filters is evident.

Integrity testing of such filters is done off-line, otherwise the solvent/water mixture used to wet the hydrophobic filter and perform the diffusion or bubble point test may contaminate the process. Tested off-line the filter is then flushed and dried, afterward installed and steam sterilized. This certainly created insecurity, because there was no assurance that the filter was integral after steam sterilization. Nowadays water-based tests, like the water intrusion or water flow integrity test are used to integrity test the filters in place after steam sterilization. As with the autoclave and lyophilizer vent filters, the filter elements can be tested fully automatically on a routine basis, preferably after every sterilization cycle.

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14 Processing of small volume parenterals and large volume parenterals

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DEFINITION OF SVPs AND LVPs

Parenterals are defined as preparations intended for injection through the skin or other external boundary tissue so that the active ingredients contained are delivered directly into the blood stream or body tissue. Parenterals are manufactured with extreme care by procedures designed to ensure that pharmacopeial requirements such as sterility, pyrogens, and particulate matter are met. Two categories of parenterals are the small volume parenterals (SVPs) and the large volume parenterals (LVPs). The term small volume parenterals applies to injections or preparations that are packaged in containers of 100 mL or less, whereas LVPs are usually intended for intravenous use and are packaged in containers of 100 mL or more (1).

CATEGORIES OF SVPs AND LVPs

Today's business world for the pharmaceutical industry shows an ever-increasing emphasis on producing products acceptable for world markets. Requirements are becoming more unified and are tending to reduce into three major pharmacopeias the United States Pharmacopeia (USP), the European Pharmacopeia (EP), and the Japanese Pharmacopeia (JP). Although fundamentally equivalent, the USP and EP define, measure, and specify different requirements at the detail level (2 4). Requirements for today's processing of parenteral products are based on validated procedures and maintained under guidelines of current Good Manufacturing Practices (cGMPs). The validated methods encompass strict controls to assure products meet pharmacopeial requirements for sterility, pyrogens, particulate matter, and other contaminants. Water used in the manufacturing of parenteral products is strictly tested, controlled, and specified to meet critical requirements for microorganism and chemical contaminants (5).

The pharmacopeia categorizes parenteral products into small and large volume parenterals based on their fill volume or their use. For example, the USP designates SVPs as containers labeled as holding a 100 mL volume or less, whereas the EP views LVPs in terms of intended use, such as infusions, solutions for irrigation, and so forth. The importance in separating definitions between large and small volume products is for purposes of specifying impurity levels associated with dosing and the sampling of individual containers for product consistency, such as particulate matter and requirements for sterilization consistency (2).

The USP further describes the definition of the Pharmacy Bulk Pack as a separate category of sterile preparation for parenteral use that contains many single doses for the specific use in a pharmacy admixture program.

Drug product form determines the next higher level of categorization. The EP lists the several categories as injections, infusions, concentrates for injections or infusions, powders for injections or infusions, gels for injections, and implants. The EP defines injections as sterile solutions, emulsions, or suspensions prepared by dissolving, emulsifying, or suspending the active substance(s) and adding excipients in water, or a suitable nonaqueous liquid or in a mixture of these vehicles (3).

Control and measurement of sterility, bacterial endotoxins-pyrogens, and uniformity of units and contents are critical quality parameters.

DOCUMENTATION MANAGEMENT

A cornerstone of good manufacturing practices (cGMPs) in the pharmaceutical industry has been good documentation practices. As stated in Title 21 CFR sections 211.100 and 211.192, "There shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess. Written production and process control procedures shall be followed ... and shall be

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documented at the time of performance. Any deviation from the written procedures shall be recorded and justified. All drug product production and control records, including those for packaging and labeling, shall be reviewed and approved by the quality control unit to determine compliance with established, approved written procedures before a batch is released or distributed."

All process and environmental control activities must be maintained and documented on a daily basis for aseptic processing operations. Review of all batch records and data is required to assure compliance with written procedures, operating parameters, and product specifications before final release of product for a given manufacturing cycle. Information in the batch record documentation includes data relating to in-process testing, environmental control, personnel monitoring, utilities [e.g., HVAC, water for injections (WFI), and steam], equipment functioning (e.g., alarms, integrity of filters), and deviations (5).

Especially relevant to aseptic processing of parenterals are the documentation practices for interventions and/or stoppages. Filling line stoppages and unplanned interventions should be recorded in the batch record noting the time and duration of the event. Interventions can increase contamination risk, and their frequency may indicate a process requiring additional controls. Written line clearance procedures, such as machine adjustments and repairs, must be established. Interventions that require substantial activity near exposed product or container closures to correct usually involve local or full line clearance. A power outage, even though brief, may affect product quality and is considered a manufacturing deviation (1).

Validation Documentation

Validation documentations are mandatory in the qualification of equipment and processes (6). These documents include user requirements specification (URS), design qualification (DQ), installation qualification/operational qualification/performance qualification (IQ/OQ/PQ), validation master plan, process validation protocols/reports, test method validation, cleaning validation, technology transfer plan, facility/process risk management assessment, and media fills/smoke studies where appropriate (5).

The URS is a critical document. For mechanical systems and software programs, the successful execution of the IQ/OQ/PQ depends upon the system expectations defined in the URS. The scope of the URS should include full details of end user operability, full details of functionality, software functionality interface, description of required system performance, performance criteria (critical parameters and operating ranges), cleaning requirements, calibration schedule, maintenance requirements, and training/documentation requirements (7). Quality must review the final set of requirements and must approve changes to any requirements that may affect the product attributes (8).

For the parenteral facility with aseptic processing, complete and rigorous validation packages are the expectation to address particle monitoring systems, isolators (media fills, smoke studies), sterilization processes (autoclavation, ETO), cleaning processes, air handling, and utilities (WFI, steam).

Electronic Document Management Systems

The implementation of compliant electronic document management (EDM) systems with process monitoring functionality, fully automated work flow, electronic batch records (EBRs) and signatures, and environmental monitoring has been slow for the pharmaceutical industry. The combination of vague regulatory agency guidance, lack of significant financial investment, extensive training, and poorly aligned technologies has impeded companies from implementing compliant EDM systems. The most recent FDA guidance for electronic records and signatures is contained in the 21 CFR Part 11 (9). In this 2003 guidance, the FDA's goal was to alleviate concerns that have been raised that Part 11 requirements would (*i*) restrict the use of electronic technology that is inconsistent with the agency's intent in issuing the ruling, (*ii*) significantly increase the costs of compliance, and (*iii*) discourage innovation and technological advances. These concerns have been raised particularly in the areas of Part 11 requirements for validation, audit trails, record retention, record copying, and legacy systems.

Pharmaceutical manufacturing facilities mostly remain on a mid-20th century platform (10). The spread of computer technology has stalled at machine-level data collection for

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tracking basic processing information. In-process samples are still taken and delivered to support laboratories and test results are delivered hours to days later. The pharmaceutical industry has lagged behind in implementing the use of IT on a large scale to automate and streamline manufacturing steps, specifically its batch record systems and for maintaining process quality control. Limited automation, paper record keeping, poor process understanding and controls, and outdated information archiving practices contribute to the industry's poor manufacturing efficiency record (11). EBR and process analytical technologies (PATs) are systems available to eliminate these inefficiencies.

EBR and PAT can create a database that can be translated into process knowledge, increased yields, and strategic-planning tools. In fairness to pharmaceutical manufacturing, reporting requirements and public safety regulations for drugs make the reliability of IT systems a critical priority. IT system crashes or glitches would likely result in lost data or process verification that could turn a million-dollar batch of medication into a company's largest quarterly loss. Laboratory information management systems (LIMSs) have emerged that are capable of addressing the complexity of the regulatory compliance and industry's best practices (12). With LIMS, data generated from an instrument electronically and then captured as a direct computer input can be identified at the time of the input by the analyst responsible for direct data entries. LIMS provides retention of full audit trails to show all changes to the data and uses timed and dated electronic signatures. The justification of changes are recorded and saved with each entry. LIMS generates final reports that automatically provide a description of the methods and materials used and a presentation of the results including calculations and statistical analysis.

MATERIALS MANAGEMENT

Materials management is the logistical planning required to ensure that sufficient raw materials, commodities, packaging components, and warehousing are available to manufacture the product and to satisfy the supply chain (13). Materials management also ensures the materials used to manufacture, package, and ship the product meet minimum quality requirements and are compliant with international standards and relevant regulatory guidelines (6).

Quality assurance for finished pharmaceuticals and medical devices includes the specification and control of those components that have product contact during manufacturing. Product contact with commodities and equipment may result in the extraction of foreign substances that may impact patient safety or compromise product potency and stability. This also includes the quality and consistency of the raw materials (excipients including salts, sugars, stabilizers, and surfactants) in the product formulation (14).

Many principles in this section are derived from international guidance for the application of appropriate GMP. This section combines existing governmental regulatory GMP principles and international quality management system requirements as developed by the International Organization for Standardization (ISO). In view of the increasing globalization of the pharmaceutical industry and the harmonization of pharmaceutical registration requirements, deference to both schemes is becoming necessary. The reach of the requirement for cGMP is moving upstream in the overall manufacturing process and in today's environment is touching on the fringe of control of excipients.

The ISO 9000 series is a quality system standard of general application that can be applied to cover every aspect of manufacturing to the benefit of both the manufacturer and the customer. It has taken several years since its introduction in 1987 for the ISO 9000 series to be utilized worldwide. Obtaining certification is a business decision as there is no current regulatory requirement in Europe, Japan, or the United States for third-party certification.

A manufacturer may apply the standard with or without certification. However, certification has the benefit of providing assurance to customers that conformance to this quality system has been independently confirmed. Incorporation of GMP requirements into the ISO 9000 quality system enhances not only the quality system, but a company's operational procedures as well. Finally, there is an increasing expectation worldwide for compliance with ISO 9002 as an essential element to qualify suppliers.

A material's qualification and control program (Fig. 1) is key to assuring drug quality, yet it is often viewed as a burdensome requirement in a busy firm and not an activity

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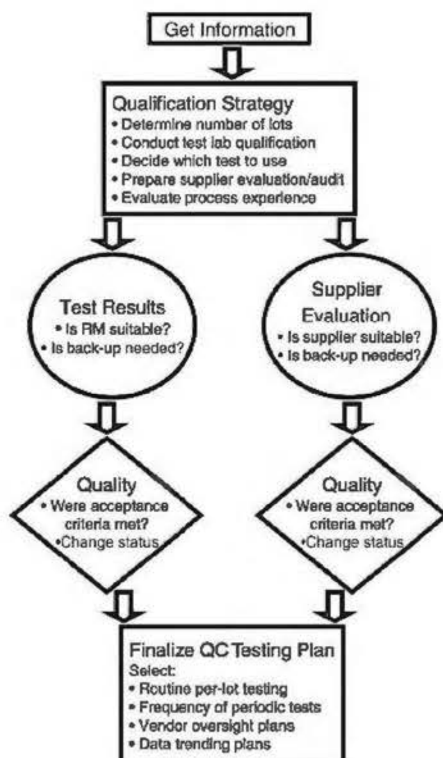

Figure 1 Material qualification and control program.

that brings process control to the product until the supply chain is derailed by a single failure (15).

GMP regulations require that pharmaceutical raw materials and their suppliers be qualified both initially and periodically (5). Similar requirements can be found in the U.S. Code of Federal Regulations (CFR), ICH guidance documents, European GMP regulations, and within ISO. Patient safety drives this requirement, dating back to several events within the pharmaceutical and food industries where unsuitable raw material led to toxicity, resulting in hallucinations and other severe symptoms (16). Mix-ups and errors of identity have also occurred.

Attention should also be paid not only to the manufacturing operation but also to how the raw material is packaged. Laboratory animal studies have reported bisphenol A to be a potential carcinogen. This leachate comes from plastic containers used in the food industry.

A pharmaceutical firm is legally responsible for the quality of the product contact materials that it purchases and uses in a cGMP manufacturing process. Consequently, it is good a business practice for a firm to oversee suppliers and test laboratories and to characterize materials appropriately (5).

Control of Excipients

It is important that manufacturers identify and set appropriate limits for impurities in excipients based on appropriate toxicological data, or limits described in national compendia as requirements, as well as sound manufacturing practice considerations. Most excipients are used in the final dosage form without further processing so all impurities obtained in the excipient generally remain in the final dosage form.

Excipients in Finished Dosage Forms

Bulk pharmaceutical excipients are required to be uniform in chemical and physical composition to assure consistent and continued final dosage-form products.

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The excipients used to manufacture commercial lots should not significantly differ from those used in clinical lot manufacture. Where significant differences do occur, additional testing by the manufacturer of finished dosage forms may be required to establish that the bioequivalence of the finished product is not adversely affected over time.

QUALITY POLICY AND CONTROL

Management should demonstrate commitment to a quality policy that should be implemented within the operational unit. Management should also participate in the development of the company's quality policy and should provide the resources necessary for development, maintenance, and review of such policy and quality systems at least annually. Management should be committed to this policy and should appoint appropriate company personnel to be responsible for coordination and implementation of the quality systems (17).

Organization

There should be a quality unit, independent of production, with the responsibility and authority to approve or reject all components, excipients, in-process materials, packaging materials, and finished drug product. The quality unit should have the authority to review production records to ensure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The quality unit should be responsible for approving or rejecting product manufactured, processed, packaged, or held under contract by another company. The quality unit can delegate these responsibilities if proper controls, such as periodic audits and documentation of training, are in place. Adequate laboratory facilities for the testing and approval or rejection of raw materials, packaging materials, in-process materials, and finished dosage form should be available to the quality control unit.

It is the responsibility of an independent unit, usually the quality assurance group, which is independent of production, to participate in issuing procedures; authorizing changes to processes, specifications, procedures, and test methods; and investigating failure and complaints.

Manufacturer and User Responsibilities

Contract Review

The manufacturer and user should mutually agree upon the specifications. The manufacturer must have the facility and process capability to consistently meet the mutually agreed-upon specifications of the product(s). Subcontracting or significant changes to a supplier's audited process that could affect the physical properties, chemistry, or functionality of the excipient in a final dosage form should be immediately communicated or preapproved as mutually agreed upon between customer and supplier.

Document and Data Control

The excipient manufacturer should have a system to control all documents and data that relate to the requirements of the quality system. Date of issue and location of these documents should be recorded. Each document should include a unique identifier, date of issue, revision number on each page, and the issuing department. All changes and the reasons for the changes should be documented. Documents and subsequent changes to the documents should be reviewed and approved by designated qualified personnel before issuance to the appropriate areas identified in the documents.

Purchasing

The purchaser should verify that the supplier of raw materials, components, and services for the manufacture of excipients has the capability to consistently meet the agreed-upon requirements. This may include periodic audits of the vendor's plant, if deemed necessary. Purchasing agreements should contain data clearly describing the product ordered, including where applicable, the following:

- The name, type, class, style, grade, item code number, or other precise identification traceable to the raw material specification

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- Drawings, process requirements, inspection instructions, and other relevant technical data, including requirements for approval or qualification of product, procedures, process equipment, and personnel

These requirements also apply to selection and control of subcontractors, which include toll manufacturers and contract laboratories.

Product Identification and Traceability

All items should be clearly identified and traceable through a documented system. The system should allow the traceability of product upstream and downstream. Identification of raw materials used in the production of processed materials should be traceable using a batch numbering system or any other appropriate system. The finished product should be traceable to the customer and retrievable in case of the need for a product recall.

Labeling

Labeling requirements for excipient packages are subject to applicable national and international regulatory requirements that may include transportation and safety measures. Procedures should be employed to protect the quality and purity of the excipient when it is packaged and to ensure that the correct label is applied to all containers. At a minimum, a good system of labeling should have the following features: the name of product, the manufacturer and distributor, a lot or batch number from which the complete lot or batch history can be determined, a file of master labels (*Note:* A designated individual should review incoming labels or labels printed on demand against the appropriate master labels), storage of labels in separate containers or compartments to prevent mix-ups, formal issuance of labels by requisition or other document, issuance of an exact number of labels sufficient for the number of containers to be labeled, retention copies, and calculated excesses, if any; reconciliation of the number of labels issued with the number of unit packages and retention labels together with the destruction of excess labels bearing lot or batch numbers; and avoidance of labeling more than one lot or batch at a time without adequate separation and controls.

There should be documentation of the system used to satisfy the intent of the previously mentioned requirements in all instances whether excipients are labeled on the packaging line, packaged in preprinted bags, or bulk shipped in tank cars.

If the need for special storage conditions exists (e.g., protection from light, heat, etc.), such restrictions should be placed on the labeling.

Retained Samples

Reserve samples of an excipient should be retained for one year after the expiration or reevaluation date or for one year after distribution is complete, whichever is longer. Sample size should be twice the amount required to perform specification testing.

EQUIPMENT CONTROL

Multipurpose Equipment

Equipment used in the manufacture, processing, packaging, or holding of a product should be of appropriate design, adequate size, and in a suitable location to facilitate its operation, cleaning, and maintenance.

Many parenterals are produced using multipurpose equipment. With few exceptions, such multiple usages are satisfactory provided the equipment can be adequately cleaned according to validated written procedures. The cleaning program should take into consideration the need for different cleaning procedures, depending on the safety considerations of the product or intermediate and what product or intermediate was previously produced. Products that leave residues that cannot be easily removed should be produced in dedicated equipment.

Where multipurpose equipment is in use, it is important to be able to determine previous usage when investigating cross-contamination or the possibility of such contamination. Methods of determining prior use include any documentation system that clearly identifies the

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previous lot or batch and shows that the equipment was cleaned. An equipment cleaning log is perhaps the most desirable and preferred method of determining prior use.

The cleaning and disinfection procedures should be properly established by competent personnel using the model product approach. These procedures should be designed to meet or exceed the particular needs of the product and process involved and should be set down in a written schedule available for the guidance of employees and management. An effective and regular cleaning program should be put in place to remove product residues and dirt that may also contain microorganisms and act as a source of contamination.

The manufacturer should demonstrate the effectiveness and efficiency of the cleaning and disinfection procedures for each piece of equipment, and the cleaning status of equipment should be recorded. Validation data ought to prove that the cleaning procedure is acceptable. An evaluation should consider the potential impact that traces of contaminant may have on the product supplied to the customer. All equipment that has been in contact with contaminated material must be thoroughly cleaned and disinfected before coming in contact with product.

Single-Use Technologies for Multiuse Production Facilities

Disposables are growing in popularity due to the large numbers of biological drugs being developed that require aseptic processes. Disposables are able to minimize cross-contamination, cleaning, start-up timeline, capital investment, production cycle, and assurance of sterility. The time saved when substituting a disposable capsule filter for a cartridge filter in stainless steel housing is presented in Table 1. When implemented, disposables, also known as single-use systems, simplify the transfer of processes across multiple sites because single-use systems are flexible, modular, and customizable. If disposables have already been designed into the biopharmaceutical process, qualification and validation are simple during technology transfers.

So what are the implications for facility design? One example is presented by considering the amount of water used for cleaning a stainless steel facility. This traditional facility design is composed of complex piping and controls for steam-in-place (SIP) and clean-in-place (CIP). If one considers a model 500 kg bulk monoclonal antibody facility of stainless steel, approximately 155,000 L of solution would be required annually for cleaning (18). However, in a disposable facility, one could:

- remove most CIP and SIP infrastructure
- remove the autoclave and washing areas
- remove process pipework between the unit operations by replacing it with physical movement and disposable tubing

The result is a facility containing clean rooms with little process infrastructure. The process is configured by setting up process operations at designated workstations that are minimally equipped with power, data links, and gases. Therefore, the operational space becomes flexible and can be easily reconfigured as desired in multiuse facilities.

Table 1 Time Comparison to Perform Filtration

Step	Presterilized capsule filter	Cartridge filter in SS housing (min)
Remove filter from packaging	5 min	5
Collect components/assemble housing	N/A	15
Autoclave filter assembly	N/A	60
Cool filter to room temperature	N/A	60
Transport assembly to process area	N/A	5
Aseptically connect filter to process train	15 min	15
Total time required	20 min	160
Time saved with presterilized filter	140 min	

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However, disposables do pose challenges. The procurement and quality teams need to perform the vendor/supplier assessments in terms of pricing, operational risk, product/chemical compatibility, and security of supply. Production will rely more on manual handling rather than pipework to move product, solutions, and materials throughout the facility. Companies will also have to address disposal options for the large volume of disposables produced by the single-use technology to be environmentally friendly as well as cost-effective.

Product Contact Material

In the course of establishing a manufacturing process, it is mandatory that the impact of materials used in the manufacturing equipment on drug product is well understood, which is also reflected in regulatory requirements (e.g., CFR Title 21, Part 211.65) (5).

At the time of implementing a manufacturing process, a complete product contact material assessment should be available. Materials used in the manufacturing process typically comprise various types of stainless steel, plastics, rubber, lubricants, and glass in the form of stainless steel tanks, plastic containers, tubing, stirrers, gaskets, valves, rings, filters, sampling devices, pumps, or fill needles. The main factors to be examined in such assessment are extractables and leachables, sorption, and chemical and physical compatibility. Consequently, a compatibility assessment of the drug and a comprehensive set of product contact materials involve exposure studies at relevant conditions where stability and sorption properties of the drug substance are monitored, along with detection of leachables from the exposed materials. In addition, extractable studies are required according to relevant guidance provided (e.g., in the USP monographs) (2). Information from prior experience, published literature, and vendors initially may be used for an assessment. However, in most cases corroboration is needed in the form of experimental data. This is especially important in the cases of biologics. The considerable complexity regarding molecule size, number of potential interaction sites, and sensitive structure/function dependence of this molecule class poses a high potential for being impacted by product contact material interactions (e.g., by leachable substance mixtures of diverse chemical nature). Therefore, it is recommended that every biologic product and its contact materials be assessed on an individual basis.

Various case studies of incidents involving leachables originating from processing equipment (i.e., stainless steel tanks, rubber gaskets, silicone tubing, and filter membranes) in commercial manufacturing have been reported in the literature (19). However, in many cases, the focus of development activities regarding material compatibility, especially in terms of leachables and extractables, is still solely on long-term storage in primary and secondary packaging containers (20,21).

Plastic Containers

Today, plastic containers are commonly used for the following types of products: LVPs, ophthalmics, otics, and inhalation therapy. The replacement of the glass container for these products has been gradual over the last few decades. A high degree of caution was based on data that showed that glass was inert and that the glass container provided a better barrier to the environment, for example, better water vapor transmission protection and better protection against intrusion of gas (22).

This early perception regarding plastics has been changing. In the case of LVPs, durability and weight savings were obvious advantages for using plastic instead of glass. However, the flexibility of plastic was also an important consideration. Plastic bags that are used to package LVP products will collapse as liquid drains out. This occurs because of the fact that the system is completely closed. Glass bottles, on the other hand, do not collapse as the fluid drains from the container and a venting system must be provided to replace the evacuated liquid with air. The venting systems have gone through a series of improvements, all of which are not as effective as the closed system provided by a plastic bag.

The blow/fill/seal technology, also known as form/fill/seal technology, is used to manufacture and fill plastic containers. This technology involves forming the container via a process known as blow molding (i.e., forming the molten plastic into a container of the desired shape) while simultaneously sterilizing the container, filling the formed sterile container with a sterile product, and then sealing the container. All of the operations are completed aseptically

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on one machine and the entire process is completed uninterrupted and in sequence. The formulated product within the container is not exposed to the surrounding environment. This eliminates the need for container inventory, washing, sterilization, and in some cases, labeling. This new technology is largely responsible for making plastic a more attractive alternative to glass for packaging SVP products.

However, the manufacturers of SVP products have been reluctant to switch from glass to plastic because plastic containers still need to be washed and sterilized like glass. In addition, the chemical sterilization methods that are required for some plastics can be more difficult to perform and less reliable than the thermal methods used to sterilize glass containers.

Nonetheless, plastic materials have some advantages for SVPs. Their higher mechanical strength can be beneficial when developing a container for the use in a device. Additionally, their modern appearance might be a marketing advantage.

LVP Films

LVPs are packaged either in glass vials with rubber stoppers or in plastic bags. Plastic bags are either delivered presterilized and ready to use with no further pretreatment before usage required, or the blow/fill/seal technology is used for manufacturing. An advantage of plastic bags over glass vials is the number of different sizes that are available and the flexible, hardly breakable properties of the materials. A high variety of assemblies with multiple connectors is available and can be customized to the special requirements of a certain application.

These plastic bags are sterilized with gamma irradiation and consist of multiple film layers of different materials such as ethylene vinyl acetate (EVA), ethyl vinyl alcohol (EVOH), and with contact to the fluid ethylene vinyl acetate monomaterial (EVAM) or layers of polyamide (PA), polyethylene terephthalate (PET), ultralow density polyethylene (ULDPE), or EVOH. The plastic film materials must comply with relevant compendial monographs for example, the monographs for "Physico-chemical test for plastics" (USP 661) or "Ethyl Vinyl Acetate Copolymer (EVA) for containers and closures" (EP 3.1.7).

For the validation of bags, testing of the films and their properties, integrity and biocompatibility, physicochemical tests, stability, chemical compatibility, bioburden, and shelf life are investigated. For example, the bags are filled with WFI, and parameters such as total organic carbon (TOC) are checked at various time points over certain time periods. Physicochemical testing according to USP 661 involves extractions with water at 70°C for 24 hours (nonvolatile residues, residue on ignition, heavy metals, and buffering capacity) or extractions with isopropanol (nonvolatile residues, residue on ignition, turbidity, and UV absorption) are performed.

For the investigation of extractables, the bag materials are "extracted" with model solvents such as water, ethanol, hydrochloric acid, or sodium hydroxide solution. The extracts are then analyzed for pH, conductivity, volatile GC-MS, nonvolatile LC-MS, and metal ICP. Additional product-specific leachable studies must be conducted and can be performed either by the bag user or by the bag manufacturer.

For the determination of protein adsorption, the bags are tested with a model protein-like bovine serum albumin or bovine IgG at different temperatures with contact times up to several days.

The evaluation of the permeation of microorganisms is performed with bags that have been aseptically filled with culture media (e.g., soybean digest casein broth) and preincubated for seven days. The bags are then immersed in challenge suspensions containing defined types and amounts of microorganisms and are afterwards incubated.

MANUFACTURING CONTROLS

Aseptic and Sterile Manufacturing

The manufacture of sterile products presents technical challenges. Since humans are the primary source of contamination in an aseptic operation, the process should be designed to eliminate this direct contact. Those aseptic operations that utilize considerable operator involvement should have adequate controls.

The manufacturer should document the sanitizing of critical processing equipment. Processes used for the sterilization of equipment should be validated. The manufacturer also should verify that no chemical interaction with the product occurs.

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There are guidelines and compliance programs that provide detailed guidance for the manufacture of sterile products (1).

Validation of Process and Control Procedures

Parenteral manufacturers are expected to adequately determine and document that all significant processing steps are performed consistently. The type of drug product, the breadth of the specification relative to the degree of process control, and the other factors determine the extent of the process development and documentation required.

An important factor in the assurance of product quality includes the adequate design and control of the manufacturing process because product testing alone is not sufficient to reveal variations that may have occurred. Each step of the manufacturing process should be controlled to the extent necessary to ensure that the product meets established specifications. The concept of process validation is a key element in ensuring that these quality assurance goals are met. Documentation describing the process reactions, operating parameters, purifications, impurities, and key tests needed for process control should be written to provide the basis for validation.

Many manufacturers already possess the data necessary to validate that their processes perform in a consistent manner. For example, limitations of a reaction or purification step are usually identified in the development phase. Known impurities and tests used to determine their levels are also established at this phase. Thus, when the process is scaled up to production of a lot or batch size, a comparison can be made with development lots or batches. Scale-up and development reports, along with purity profiles, would constitute an appropriate validation report.

In-Process Testing

Parenteral products are normally subject to various in-process tests to show that a manufacturing process is proceeding satisfactorily. Such tests often are performed by production personnel in production laboratory facilities. Approval to continue with the process is often issued within the production department. The important considerations are that specified tests be performed and recorded by trained personnel and that the results are within specified limits.

In-process inspection and testing should be performed based on monitoring the process or actual sample analysis at defined locations and times. The results should conform to established process parameters. Work instructions should delineate the procedure to follow and how to utilize the inspection and test data to control the process.

Finished Product Testing and Release

Finished product testing should be performed by the quality unit and should conform to written specification. There should be a procedure that ensures prior to release that the evaluation of the appropriate manufacturing documentation and test data occurs.

All appropriate records relating to inspection and testing must be available for review. Where the process is continuously monitored, acknowledgment that the process was monitored and the results of the monitoring should be available.

Control of Nonconforming Product

Any raw material, intermediate, or finished product found not to meet specifications should be clearly identified and segregated to prevent inadvertent use or release for sale. A record of nonconforming product should be maintained. All incidence of nonconformance should be investigated to identify the root cause. This investigation should be documented and corrections made to prevent recurrence of the problem.

Procedures should exist for the evaluation and fate of nonconforming products. Nonconforming products should be reviewed in accordance with documented procedures to determine final outcome.

Inspection, Measuring, and Test Equipment

Calibration of all in-process and laboratory instruments, identified as quality instrumentation, should be traceable to recognized standards. The control program needs to include the

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calibration of reagents, instruments, apparatus, gauges, and recording devices at suitable intervals in accordance with an established written program containing specific directions, schedules, limits for accuracy and precision, and provisions for action in the event accuracy or precision limits are not met. Equipment not meeting established specifications should not be used.

Computer systems used to verify that the product conforms to specifications should be audited to ensure satisfactory performance.

Quality Record Control

The manufacturer should establish and maintain procedures for identification, collection, indexing, filing, storage, maintenance, and disposition of quality records. Quality records should be maintained to demonstrate achievement of the required quality and the effective operation of the quality system. Pertinent subcontractor quality records should be an element of the data.

Quality records should be kept for at least as long as samples are retained or in accordance with legislative requirements. These records should be stored in facilities that provide a suitable environment to minimize deterioration or damage and to prevent loss and should be maintained in such a way that they are readily retrievable.

Batch production and control records should be prepared for each batch of drug product produced and should include complete information relating to the production and control of each batch. These records should include an accurate reproduction of the appropriate master production record, checked for accuracy, dated, and signed as well as documentation that each significant step in the manufacture, processing, packing, or holding of the batch was accomplished.

Internal Quality Audits

The parenteral manufacturer should carry out a comprehensive system of planned and documented internal quality audits to verify whether quality activities comply with planned arrangements and to determine the effectiveness of the quality system. Audits should be scheduled on the basis of the status and importance of the activity. The audits and follow-up actions must be carried out in accordance with documented procedures. The results of the audits should be documented and brought to the attention of the management personnel having responsibility in the area audited. Personnel responsible for the area should take corrective action on the deficiencies found by the audit. Quality risk management (e.g., FMEA analysis) should be implemented to reduce future risks, focus validation efforts, and maximize the business value of the manufacturing process (23).

Training

The parenteral manufacturer should establish and maintain procedures for identifying and providing the training needs of all personnel performing activities affecting quality. Appropriate records of training should be maintained. Training should directly relate to the employee's function or performance of specific operations and to GMP. This training should be conducted by qualified individuals on a continuing basis and with sufficient frequency to ensure that employees remain familiar with any applicable manufacturing practice requirements.

PROCESSING OF COMPONENTS

Siliconization

When manufacturing parenteral products, various parts of the primary packaging materials can be siliconized with polydimethylsiloxane (PDMS, e.g., Dow Corning DC-360, Medical Fluid). Examples are as follows:

- Vials
- Syringe barrels
- Stoppers
- Syringe needles

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Silicone oil is applied on glass containers to mask glass surfaces by sealing microcracks. Also, siliconization facilitates complete emptying of syringes or vials, which in turn will decrease the loss of drug due to residual volumes in the containers and leads to a reduction of the required overfill volumes.

For syringes as primary packaging material, siliconization is needed to enable stopper movement in the syringe. Unsiliconized syringe barrels cannot be emptied due to high gliding forces. The quality and quantity of siliconization determines the ejection forces and are therefore crucial for the functionality of the syringe, which is especially important in autoinjector devices. Syringe forces are divided into categories of the breakout force and the gliding force. The breakout force is the initial force needed to start the stopper moving, while the gliding force is defined as the force needed to keep the stopper traveling to the end of the syringe barrel.

Syringe forces can be controlled by defining the type and amount of silicone oil applied. The higher the viscosity of the oil, the higher the forces. Forces can be decreased by decreasing oil viscosity and/or increasing the amount of silicone oil per syringe. The amount per syringe must be chosen so that it is compatible with the respective drug and does not detach from the syringe walls over time within the chosen storage time and temperature. In general, lower viscosities are preferred since the distribution is more even, resulting in smoother gliding forces and fewer areas with insufficient siliconization.

Stopper siliconization mainly improves processability during manufacturing by supporting stopper insertion and by preventing the stoppers from sticking together due to the high friction of the rubber. Syringe needles are siliconized to facilitate needle insertion into the skin and to reduce the sensation of pain.

PDMS has a molecular weight of 1000 to 150,000 Da and a viscosity of 10 to 107 mPa·s and can be applied to the glass surfaces as pure oil or as an emulsion. As an oil, it is applied via two methods, either as a wipe-on siliconization with silicone-soaked fabric or O-rings or with spray-on siliconization through nozzles. Under standard conditions no binding or polymerizations of the silicone occurs. For the emulsion, 1% to 3% PDMS with WFI and an emulsifier (e.g., polysorbate 20) are stirred until a stable, homogenous emulsion is obtained. Vials or syringes are immersed into the oil-water emulsion and are then heated to 250 to 300°C to generate covalent bindings (Si O Si), and 1 to 10 layers are fixed to the surface mostly as free silicone. Water and Emulsifier are removed with heat during that procedure, which is called "baking" of the silicone onto the glass surfaces.

The applied amount of silicone can be controlled by reading the scale of the tank display (consumed amount of oil per batch) or by analysis of the individual syringe or vial: control of pump movement and compressed air during application of the oil per unit; weighing before and after silicone application; extraction with Toluol (destructive); or via FTIR, Raman, or refractometry as nondestructive tests. For prefillable syringes, the quality and quantity of siliconization can be determined indirectly by force measurement (destructive). In general, the maximum breakout forces and gliding forces should be specified on the level of the empty syringes to avoid product failures with filled product. The average gliding force is a measure for the amount of silicone oil applied, and the profile is a measure of the uniformity of the silicone film. The more uneven the gliding force, the less uniform the distribution. Uniformity of siliconization is especially important when using an autoinjection device since these are usually spring driven and can only deliver a defined force over the barrel length.

Washing of Vials, Stoppers, Hoses, Pump Assembly, and Tanks

For the aseptic manufacturing of SVPs, all manufacturing equipment and primary packaging materials must be clean and sterile. Presterilization preparation of manufacturing materials usually involves a series of wash and rinse cycles to remove foreign particulate matter and to reduce bioburden as well as endotoxin load. The quality of water to be used is defined in FDA and European Medicines Agency (EMEA) guidelines and must comply with the monograph for "Purified Water" for first rinsing and washing and the monograph "Water for Injectable Products" for the final rinsing step for parenteral product equipment.

The use of detergents should be avoided, if possible, since residues could be hard to eliminate and as a result may contaminate the product.

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The FDA recommends an area classified with a Class 100000 (ISO 8) air cleanliness level appropriate for less critical activities such as equipment cleaning.

The time between washing, drying, and sterilizing should be minimized since residual moisture can support microbial growth and the generation of endotoxins.

In addition, equipment should be designed to be easily assembled, disassembled, cleaned, sanitized, and/or sterilized. Pieces of equipment that are hard to disassemble or clean, such as tubings and fill needles, might be defined as single use to avoid costly cleaning validation.

Clean in Place

CIP is a method of cleaning the interior surfaces of pipes, vessels, process equipment, and associated fittings, without disassembly. Some CIP procedures employ initial rinses with appropriate high-purity water and/or cleaning agents followed by final rinses with heated WFI. The washing process consists of several cycles in which rinsing material is recycled through the vessels, pumps, valves, and other process equipment in a flow system. The cleaning end point is measured by analytical instruments that monitor the composition of rinse water.

Sterile in Place

Using SIP technology, the amount of aseptic manipulations can be reduced by sterilizing the preassembled connection of hoses, pipes, and tanks after CIP. The installation must be capable of withstanding steam pressure up to approximately 20 psi and sterilizing temperatures of 121 to 125°C. Furthermore, the whole system must be validated. Steam must be able to reach all parts of the equipment that have product contact for sufficient duration. Temperature sensors and pressure must be installed to monitor data during the sterilization cycle.

Some materials (e.g., rubber stoppers) are also available prewashed "ready to sterilize" or even already sterile "ready to use." This reduces the number of operations and risk of contaminations during the preparation steps. In addition, the components may be used immediately without additional operations, and investments and validations for washing and sterilizing equipment are decreased. Filling equipment can be different if "ready to use" materials are employed.

Depyrogenation and Sterilization

Depyrogenation of equipment surfaces, glass, and metal parts can be attained by high-temperature dry heat. For temperature-sensitive parts such as rubber stoppers and hoses, depyrogenation is achieved by multiple cycles of washing and multiple rinses of hot WFI prior to final steam sterilization (autoclaving), gas sterilization by ethylene oxide, or gamma irradiation sterilization.

COMPOUNDING SOLUTION

For the majority of LVPs and many SVPs, the compounding of parenteral bulk drug product involves simple dissolution of soluble ingredients in WFI. This generally straightforward process, however, is complicated by the high level of cleanliness that must be imposed to minimize the risk of product contamination by extraneous particulate matter, viable organisms, or pyrogenic substances.

Parenteral solutions typically contain soluble active ingredients next to several excipients such as osmotic adjusters, buffering agents, and (if required) bacteriostatic agents. The usual practice for compounding is to fill the tank with the larger part of the required volume of WFI and then to add the ingredients with agitation.

To ensure complete dissolution of even hard-to-dissolve ingredients or very concentrated solutes within a practical period of time, high temperatures and high mixing shear may be applied if the stability profiles of components allow. Special preparations for parenterals (e.g., suspensions, oil-in-water emulsions, cosolvent systems, and nonaqueous systems) may require shear-intensive dispersion and homogenization operations. Jacketed mixing tanks with both an inner and an outer wall are used for heating and subsequent cooling of the product solution. For heating and cooling, steam and cooling liquid, respectively, are admitted into the

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space between the tank walls. The mixing process and mixing pattern in a stirred tank is defined by a number of parameters, such as tank geometry, mixing speed, eccentricity of the mixer, and mixer type. On the basis of required mixing efficiency and allowable shear, a mixer geometry producing a radial, axial, and tangential flow pattern, respectively, is chosen. Commonly used systems include top-mounted impeller or paddle, magnetically coupled bottom impeller, or stir bar. To further increase mixing efficiency and avoid vortex formation, baffles (i.e., static elements mounted radially at the tank wall) can be added. In general, the formation of a vortex in the liquid is to be minimized during mixing because it may lead to centrifugation with minimal mixing efficiency and potentially, to severe air entrainment.

After dissolution is complete, the preparation pH is checked and adjusted if required. The bulk preparation is brought to final volume with WFI and is mixed.

An increasingly important exception to this general process is represented by the group of biotech parenteral drugs (i.e., monoclonal antibodies). Here the bulk drug substance is commonly produced as aqueous liquid solution that has the same composition as the final product. The liquid state may be suitable for short-term holding. However, due to the benefits of increasing product stability, extending shelf life, and decreasing potential for microbial growth, the biologic bulk drug substance is preferably stored long term and shipped in a frozen state. Several platform technologies based on stainless steel tanks or disposable containers (e.g., bottles, carboys, and bags) have been developed for this purpose. All systems require a thawing step for the bulk drug substance before subsequent unit operations of the compounding process occur. Depending on the selected freeze and thaw system, additional low-shear mixing and dilution with a compounded excipient solution may be necessary.

FILTRATION

Filtration is a common method of sterilizing drug product solutions. A sterilizing-grade filter should be validated to reproducibly remove viable microorganisms from the process stream, producing a sterile effluent. Currently, such filters usually have a rated pore size of 0.22 μm or smaller. Use of redundant sterilizing filters should be considered in many cases. Whatever filter or combination of filters is used, validation should include microbiological challenges to simulate worst-case production conditions for the material to be filtered and integrity test results of the filters used for the study.

Factors that can affect filter performance generally include (*i*) viscosity and surface tension of the material to be filtered, (*ii*) pH, (*iii*) compatibility of the material or formulation components with the filter itself, (*iv*) pressures, (*v*) flow rates, (*vi*) maximum use time, (*vii*) temperature, (*viii*) osmolality, and (*ix*) the effects of hydraulic shock. When designing the filter validation protocol, it is important to address the effect of the extremes of processing factors on the filter capability to produce sterile effluent. Filter validation should be conducted at maximum filter use time and pressure.

It is essential that laboratory experiments model actual production conditions. A production filter's integrity test specification should be consistent with data generated during microbial retention validation studies. Sterilizing filters should be discarded after processing of a single lot (1).

A filter validation package should be updated when modifications are implemented that impact the filtration step(s) in the manufacturing process. These may include changes to filter device or membrane composition, filter contact time, batch size, solution formulation, temperature, flow rate, and pressure. A careful review of the microbial retention challenge filtration process conditions and the solution volume and properties is required to determine any gaps created by the change(s). A typical assessment is presented below.

Case Study: Batch Size Scale-Up Rationale for Filter Revalidation of Microbial Retention Challenge

Issue

The current filter validation for bulk solution supported 32L batch size filtered through 1000 cm^2 filter device based on microbial retention and filter device extractables. Production plan was to increase batch size to 130L while maintaining 1000 cm^2 filter device. No changes were made to the filter membrane, pore size, or product contact materials. Is microbial retention challenge revalidation recommended for the filtration step?

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Rationale

A scaled-down batch volume of approximately 700 mL was filtered in the previous microbial retention challenge through a 13.8-cm^2 effective surface area membrane. Upon meeting the acceptance criteria of no detected bacterial growth in the filtrates ($n = 3$), the 700-mL scaled-down volume justified the filtration of bulk solution up to a maximum 50L batch size when using the 1000-cm^2 membrane surface area.

$$\text{Scaled-down batch volume (mL)} = \frac{\text{Maximum process batch volume}}{\text{Surface area of process filter (cm}^2\text{)}} \times \text{Surface area test filter (cm}^2\text{)}$$

$$700\,\text{mL} = \frac{\text{Maximum process batch volume}}{1000\,\text{cm}^2} \times 13.8\,\text{cm}^2 = 50,700\,\text{mL}$$

A scaled-down batch volume of 1794 mL is required for a 130L batch size using a 1000-cm^2 membrane surface area. The minimum normal flow volume processed during the revalidation microbial retention test for bulk solution was 2281 mL, which exceeded the scaled-down batch volume of 1794 mL required by calculation for the 130L batch size when using a filter device with 1000 cm^2 membrane surface area.

Therefore, the future 130L batch size processed using a 1000-cm^2 filter device will satisfy the quality requirements and objectives of the filter microbial retention challenge revalidation if the excipients and potency of the formulation, the flow rate, the pressure, the filter contact time, and the process temperature remain within current operating ranges.

DISPENSING/FILLING

The purpose of the dispensing step is to subdivide the bulk drug solution into individual container fills and to transfer these doses into the individual primary container. Commonly, the dispensing is performed in-line just after the final filtration.

Many fill systems rely on volumetric displacement pumps consisting of a cylinder and piston assembly. A fixed volume of fluid is hereby drawn into the pump chamber and then discharged into the primary container. The adjustable piston stroke determines the dispensed volume. However, in recent years, alternative pump technologies (e.g., time pressure filling, rolling diaphragm pumps, mass flow filling, and peristaltic pumps) have become more popular. This trend has appeared partly because of certain incompatibilities of the piston pumps with biotech products, which in some reported cases led to protein particle contamination in the filled containers.

In general, the dispensing step is to be considered critical for parenteral product quality and safety. This is mainly due to the potential impact of the dispensing step on fill volume precision and the increased contamination risk associated with procedures handling open product and open containers.

The fill volume precision of the dispensing step is critical to the dosing accuracy at delivery. The required precision and allowable variability of the delivered dose is generally determined by the clinical safety and efficacy data, that is, the therapeutic window of the drug, regulatory filings, specifications, and the level of required and feasible process control. The latter, in many cases, provides the most stringent requirements to fill parameters. The challenge for the manufacturer might be to find a realistic balance between manufacturing throughput for an effective use of production time and the level of fill volume precision and variability that exceeds the baseline of clinical, quality, and regulatory requirements.

> It must be noted that only the total nominal fill volume can be controlled by the dispensing step. However, the therapeutically relevant parameter is the extractable volume out of a given container according to compendial methods.

To ensure delivery of the labeled dose, overfill in addition to the target dose volume is required in the primary container. The excess fill accounts for nominal volume losses due to dead volumes in the container and delivery system (i.e., liquid volume that will remain inside

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the system after the application is completed). For example, a prefilled syringe has only minimal dead volume between the stopper end position and the needle tip (24). For an infusion, the overfill needs to be sufficient to fill the intravenous set and provide for the undrained residue in the container. General guidance regarding the amount of excess volume is provided in the pharmacopeias.

In addition to system losses, the overfill volume is also influenced by the statistical variability of the fill process. In general, the more variable the fill volume, the larger the necessary overfill. The manufacturer might be tempted to focus solely on the optimization of the average fill volume, which is accessible more easily than the distribution. However, by means of statistics, the fill volumes of the individual containers will be more or less broadly distributed around the average fill volume. The extractable volume requirement is directed toward individual fills and not the average volume over a number of containers. Therefore, the added overfill needs to not only ensure sufficient extractable volume from a container filled with average volume but also for individual container fills that reside at the lower end of the fill volume distribution.

The filling into primary containers also presents an elevated potential for extraneous product contamination. In most cases, the dispensing requires the handling of open primary containers and open product solution. This increased exposure risk is aggravated by the fact that contamination originating from this part of the process cannot be removed by subsequent filtration downstream because final bulk filtration is usually performed before dispensing. From a process capability and risk management standpoint, this scenario is especially undesirable because, to avoid administration to the patient, the manufacturer has to solely rely on inspection and quality control mechanisms that will be able to function as safeguards to identify and reject the contaminated units or batches.

Nonetheless, the most stringent precautions with regards to risk of microbial contamination are to be applied to the manufacturing of aseptically filled preparations that are not terminally sterilized. Liquid formulations of biologics are typically sterilized by filtration and aseptically filled into vials or syringes. In a worst-case scenario, contamination of this type of product after sterile filtration can potentially lead to viable organisms residing and under circumstances growing in the product container, which if undetected, is to be considered a critical situation for patient safety.

For the dispensing of various parenteral products, special considerations for the fill process may be necessary. Colloidal disperse systems (e.g., suspensions) require additional attention to maintenance of uniformity through adequate mixing and/or recirculation during subdivision.

Lyophilization

Freeze-drying (lyophilization) is a drying process used for the manufacture of pharmaceuticals, biologicals, serums, and hormones that are thermolabile or otherwise unstable in aqueous solution for prolonged storage periods, but that are stable in the dry state (17). Additionally, lyophilization can improve the dissolution properties of hardly soluble compounds.

By removing the solvent by the physical process of sublimation, heat-sensitive drugs or biologicals can be dried with a minimum of degradation of product. Degradation is minimized by (*i*) reduction of heat input during drying and (*ii*) avoidance of prolonged solution of the drug in liquid solvent during the solvent removal phase. By comparison, an evaporative process requires heat to remove the solvent. The evaporative process continuously makes a more concentrated solution. These two factors can accelerate the degradation kinetics.

Drug product bulk materials for freeze-drying are prepared and sterilized as sterile solutions or sterile suspensions and are filled into containers. Most commonly, glass vials are used, but other containers such as bags are also available. Prior to placing the vials into the chamber, special closures (Fig. 2) are loosely placed into the necks of the vials. The slots in the closures allow solvent vapor to escape from the vials during the drying cycle.

Freeze-drying consists of the three steps: (*i*) freezing, (*ii*) primary drying, and (*iii*) secondary drying. After the desired amount of material is filled into a container, the container is subjected to freezing, and then the drying process is commenced. The product should be cooled to a temperature below its eutectic point (18) and is subjected to extremely

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Figure 2 An example of a special closure on the neck of a vial with lyophilized drug.

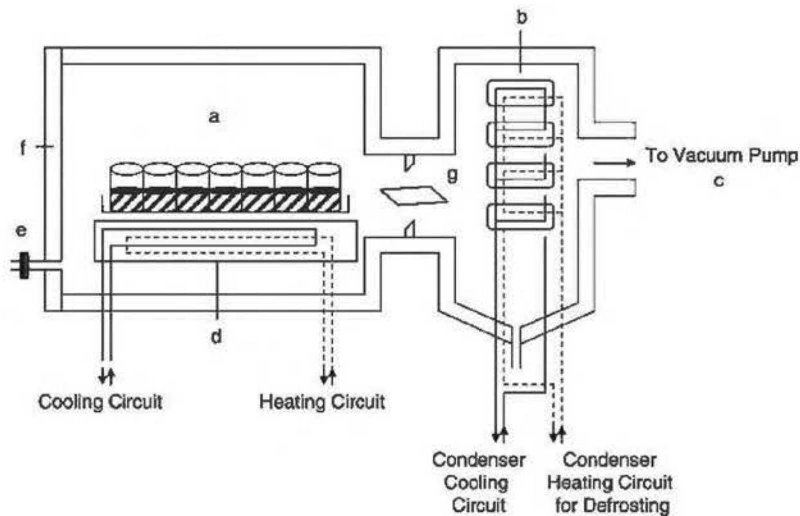


Figure 3 Schematic of a freeze dryer: (a) Drying chamber, (b) condenser, (c) vacuum pump, (d) heating and cooling shelving, (e) aeration valve, (f) loading/unloading door, (g) isolation valve.

low pressures. Under these conditions, the frozen solvent sublimates from the solid directly to the gaseous state. During primary drying, most of the solvent is removed and a "cake" is formed. During secondary drying, which usually requires a small amount of external heat energy input, vestigial solvent is eliminated.

A freeze dryer consists of a drying chamber with shelf space for the vials, a condenser for the sublimation of solvent, a pump for vacuum generation, and an electronic controller equipment (Fig. 3). For the manufacturing of parenteral formulations, the drying chamber is usually accessible from the aseptic working area.

The shelf space consists of metal plates with integrated cooling and heating circuits for freezing, cooling, and heating of the product.

Production-sized freeze driers are usually operated by an automatic control system. The temperature of a sample of the product is continuously monitored throughout the process. The temperature of the sample will steadily drop if no heat is introduced into the system because the vaporization of the solvent results in a removal of heat from the product. Therefore, after equilibrium has been reached, it becomes necessary to introduce heat into the system at a controlled rate. By monitoring the temperature of the sample, the rate of introduction of heat into the system is controlled in comparison with the rate experimentally found to produce a satisfactory product.

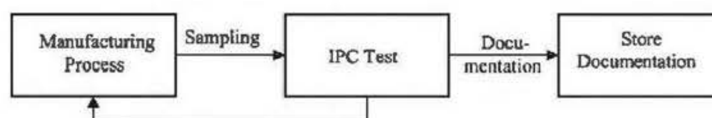
After the drying process is completed, the vials are sealed as rapidly as possible to prevent any sorption of moisture. Some freeze driers are equipped with a mechanism to press the rubber closures firmly into the neck of the vials prior to removal from the chamber.

In-Process Testing

In-process controls comprise all controls performed during a manufacturing process to monitor and control the process to obtain a product within its predefined specification. A sampling plan with the sampling points, number of samples, sampling frequency, place of sample analysis, sampling containers, acceptance criteria, and purpose of the test must be approved and in place before any manufacturing campaign. Samples should be statistically and/or scientifically representative for the manufacturing process.

Sample pull is performed as defined, and samples are either delivered to the appropriate departments or analytics are performed in the manufacturing area and results are documented as described in the sampling plan and SOPs. The results are then checked and the process is either followed as before, if the results are within the specifications, or adapted accordingly. Manufacturing processes can either be interrupted for IPC testing or testing can be performed in parallel and must be defined in the respective manufacturing instructions. Sampling equipment must be suitable and clean.

All steps of the manufacturing process are monitored with in-process control samples: preparation of excipient or bulk solution (e.g., temperature of WFI, pH, density, and osmolality of the solution), filtration pressure, hold times, and filling parameters such as fill volumes (vials and prefilled syringes) and stopper positions (for prefilled syringes). Parameters investigated also include environmental monitoring, for example, room temperature, pressure, humidity, and status of particles in the filling areas. In addition, microbial monitoring of the personnel, manufacturing area, and filling equipment is conducted.



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15 | Freeze-drying: principles and practice

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INTRODUCTION

Freeze-drying, or lyophilization, is a critical unit operation in pharmaceutical development and manufacturing because it allows removal of water from heat sensitive materials at low temperature, thereby avoiding thermal damage caused by more traditional drying operations. Freeze-drying is most important in production of injectable pharmaceuticals, but also finds application in manufacture of diagnostics and in certain solid oral dosage forms where rapid disintegration and dissolution is critical. In addition to allowing removal of water at low temperature, freeze-drying under appropriate conditions generally results in a solid material with a relatively high specific surface area, which facilitates rapid, complete dissolution. This is a critical quality attribute for drugs administered in emergency situations. Finally, freeze-drying is more compatible with sterile operations than filling a solid powder into vials. The solution can be sterile filtered immediately before filling, and fill weights of liquids dispensed into the primary container can be more consistently controlled than filling of dry powders. Filling of a solution into vials also avoids potential problems with cross-contamination through airborne particulate matter, as well as exposure of workers to potentially hazardous drugs.

Freeze-drying as an industrial process was introduced at around the time of World War II for production of freeze-dried human plasma, followed by manufacture of antibiotics, steroids, and injectable vitamins. The application of freeze-drying to manufacture of injectable products has continued to grow, particularly with the advent of biotechnology-based therapeutics. According to data from the Pharmaceutical Research and Manufacturer's Association, at least 165 biotechnology-derived therapeutic agents have been approved since the first such product, Humulin®, was approved in 1982. As of the end of 2008, there were 663 new medicines in development. Of these, 223 were vaccines, 192 were monoclonal antibodies, and 66 were recombinant proteins. While certainly not all of these dosage forms will be freeze dried, proteins are often either chemically or physically unstable in solution. This makes freeze-drying an essential step in the manufacture of many of these products.

Freeze-drying takes place because of sublimation, where water converts from the solid state to the vapor state without first becoming a liquid. This can only occur below the *triple point* of ice, which is at a temperature of about 0°C and a pressure of about 4.5 mmHg, or 4.5 torr. This pressure refers to the partial pressure of water vapor, not the total system pressure, so sublimation of ice can take place at atmospheric pressure as long as the partial pressure of water vapor is less than about 4.5 torr. Everyday examples of sublimation of ice include ice cubes shrinking over time in the home freezer, as well as "freezer burn" caused by local sublimation in frozen food products. However, these processes are very slow. To be a practical process commercially, the system pressure must be maintained below the vapor pressure of ice, so that water vapor is transported by bulk vapor flow from a region of high pressure (the ice surface) to a lower pressure maintained in the freeze dry chamber by means of a condenser operating at temperatures in the range of 60 to 80°C.

Freeze-drying has some important limitations. Often the physical state of a drug; that is, whether it is crystalline or amorphous, is critical in determining its stability as a solid. If a drug does not crystallize from a freezing system, and the amorphous solid is too unstable to provide an acceptable shelf life, then freeze-drying may not be feasible. In addition, freeze-drying is an inefficient and expensive process, both in terms of capital cost and operating expenses. This arises primarily from the high heat input required to sublime the ice (about 2800 J/g) and the fact that heat must be applied to an evacuated system, making for very poor heat transfer. Therefore, it is important for pharmaceutical development scientists and engineers to develop processing conditions that maximize efficiency and avoid consuming freeze-drying plant capacity unnecessarily.

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The purpose of this chapter is to review the scientific and engineering principles important to freeze-drying and to present an overview of practical considerations important to both formulation development and manufacture of freeze-dried parenteral products.

PROCESS OVERVIEW

Nearly all freeze-dried injectables are aseptically processed (as opposed to being terminally sterilized), where the required volume of liquid is filled into previously washed, sterilized, and depyrogenated glass vials. Special elastomeric closures with slots allowing the escape of water vapor (called a "lyostopper", see Fig. 1) are then partially inserted into the neck of the vials, and the vials are transported from the filling/stoppering line to the freeze dryer. Many modern production operations avoid placing vials in trays with a bottom because this introduces variability in heat transfer. Instead, either removable tray bottoms are used when trays of filled vials are transported manually or an automatic loading/unloading system is used. In either case, vials sit directly on the shelf of the freeze dryer.

The basic features of a freeze dryer are shown schematically in Figure 2. The basic components of the freeze dryer are a heat transfer system for removing and applying heat to the product, a condenser to collect the water vapor from the product, and a vacuum system. The shelves of the freeze dryer contain internal channels allowing the flow of a heat transfer fluid, usually silicone oil, to control the temperature of the shelf. Freeze dryers for injectable products also have sterilization systems for the chamber and condenser, and commonly have internal spray nozzles to clean the system in place.

It is general practice during development of a freeze-dried injectable to place a temperature-measuring device (usually a thermocouple) in several vials of product to monitor the status of the product throughout the process (Fig. 3). The product is first frozen to a low enough temperature to allow complete solidification of the product. The chamber is then evacuated to a pressure lower than the vapor pressure of ice (Table 1). For example, the vapor pressure of ice at 40°C is about 96 millitorr (mT). In order for sublimation to take place at an appreciable rate, the chamber pressure must be lower than this pressure. After the required pressure is reached and the condenser is cooled, heat is applied to the shelves to provide the heat of sublimation of ice. This phase is called *primary drying*, where ice in the frozen material sublimes and flows through the porous bed of partially dried product into the headspace of the vial, out the open slot in the lyostopper, and ultimately collecting on the condenser. Primary drying is characterized by a visible sublimation front that recedes from the top to the bottom of the frozen layer. Product temperature usually increases at a slow rate during primary drying, since the heat supplied by the shelf provides the heat of sublimation of ice. When primary drying is complete, the process is usually not over because, in most real formulations, not all of

Figure 1 Freeze dried products use a special stopper with a slot that is open when the stopper is in the partially seated position to allow escape of water vapor. The stack of shelves in the freeze dryer is compressed at the end of the cycle to force the stopper into its fully seated position.

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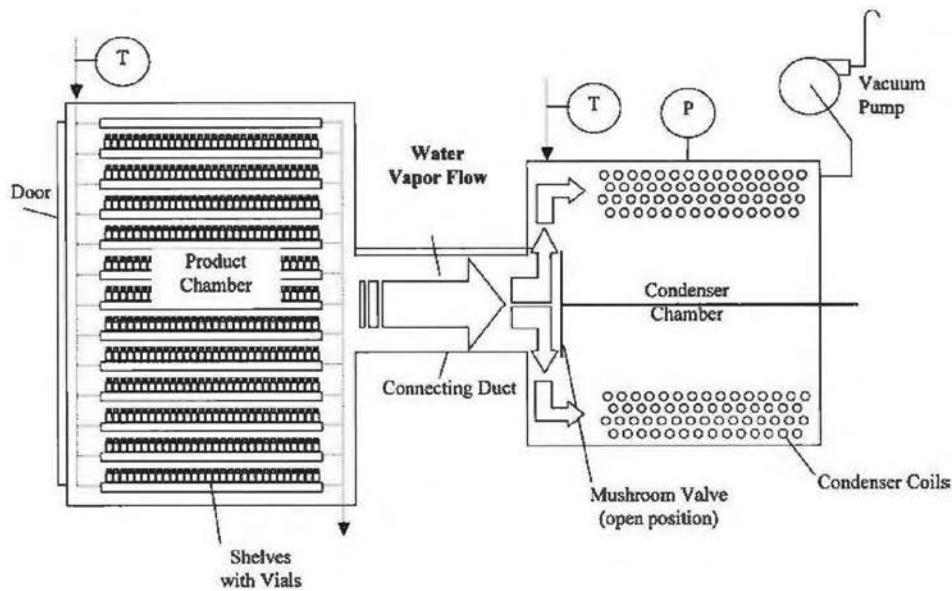

Figure 2 Schematic of a pharmaceutical freeze dryer.

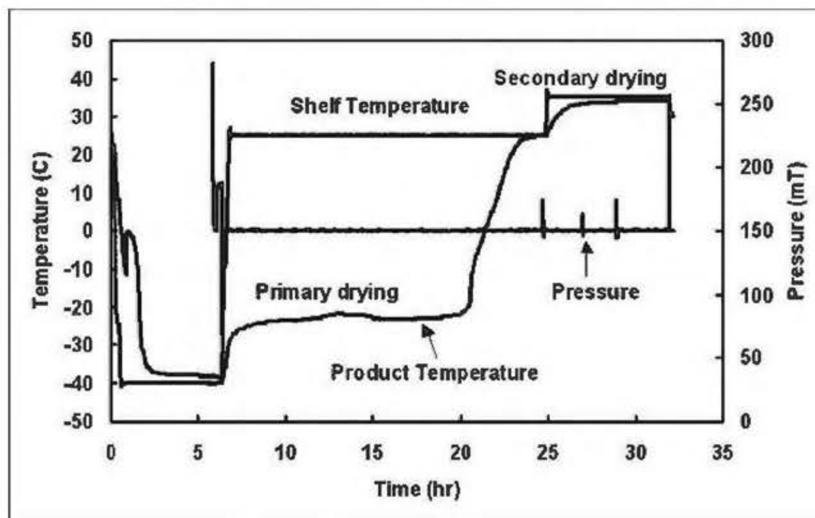

Figure 3 Primary and secondary drying stages of a freeze dry cycle as indicated by the product temperature.

the water freezes. This unfrozen water is removed during *secondary drying*. In secondary drying, ice is no longer present to use the energy provided by the shelf, and the product temperature increases relatively rapidly toward the shelf temperature. As the secondary drying process ends, the product temperature approaches a steady-state value near the shelf temperature.

When the product is sufficiently dry, the stoppers, which were partially inserted after filling the vials, are inserted into the completely seated position by means of a hydraulic

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Table 1 Vapor Pressure of Ice

Temperature (°C)	Pressure (torr)	Temperature (°C)	Pressure (torr)
2	4.58	26	0.430
4	3.86	28	0.351
6	2.76	30	0.286
8	2.33	32	0.232
10	1.95	34	0.187
12	1.63	36	0.151
14	1.36	38	0.121
16	1.13	40	0.096
18	0.939	50	0.029
20	0.776	60	0.009
22	0.640	70	0.002
24	0.526		

system that compresses the stack of shelves. It is common practice to stopper the vials while the chamber is still under at least partial vacuum, which aids in seating the stoppers and facilitates reconstitution. It is common to "backfill" with nitrogen or another inert gas prior to fully seating the stoppers.

The most important objective in developing a freeze-dried product is to assure the quality requirements are met not only initially but throughout the shelf life of the product. These quality attributes include complete recovery of the activity of the product after addition of water (called *reconstitution*), reconstitution time, freedom from extraneous particulate matter, sterility, the absence of pyrogens, and residual water content. In addition, however, process conditions should be chosen to maximize process efficiency. Freeze-drying often requires two to three days from the start of freezing to the completion of secondary drying. Success in meeting the quality requirements of the product as well as minimizing inefficiencies in the process requires a good understanding of formulation of freeze-dried products, physical chemistry of freezing, the principles of heat and mass transfer, and process monitoring. These topics will be covered in more detail below.

FORMULATION OF FREEZE-DRIED PRODUCTS

A good general rule in developing a formulation of a freeze-dried pharmaceutical product, or any product for that matter, is to keep the formulation as simple as possible, and to not include any component without a clear rationale for doing so along with supporting data. It is important to have a clear idea of the critical quality attributes of the product before beginning. Some attributes are obvious, such as sterile, nonpyrogenic, and compliant with compendial requirements for visible and subvisible particulate matter. Complete recovery of the activity present in the formulation prior to freeze-drying is always desired but may not always be possible. Vaccines, for example, tend to lose some potency as a result of freeze-drying, but the critical factor here is consistency of activity in the reconstituted solution. Dissolution of the freeze-dried cake should be complete, and the reconstitution time should be as fast as possible. Some quality attributes may, or may not, be critical depending on the intended route of injection. For example, it is always desirable for a formulation to be isotonic (the same osmotic concentration as normal physiological fluid). However, this attribute is only critical for certain routes of administration such as intraspinal, intraocular, or into any part of the brain. The same consideration applies to the pH of the formulation, where it is always desirable to have the formulation pH the same as normal plasma, but the reality is that the pH of injectable formulations varies widely as required to achieve suitable solubility and stability in solution. This applies to antimicrobial preservatives as well, but their presence in freeze-dried formulations is rarely justified.

Preformulation Considerations

The amount of drug per vial for freeze-dried products may vary from a few micrograms to two or more grams. At low doses, bulking agents are used so that the drug is uniformly dispersed

in a pharmaceutically acceptable solid matrix, in which case the freeze-drying characteristics of the formulation can be determined by the bulking agent. High doses can be more challenging, since the drug will probably determine the freeze-drying characteristics of the formulation. The total amount of dissolved solids should not be too low, or too high. If the dissolved solids content in the pre-freeze-dried solution is too low, the appearance of the cake may not be acceptable and, more importantly, the dried solids may be so friable that powder is ejected from the vial during primary drying. If the dissolved solids concentration is too high, this may lead to difficulty in process control if the resistance of the dried product layer to the flow of water vapor is too high, where a high resistance is indicated by a rapid increase in product temperature during primary drying. As a very rough guideline, the formulation scientist should aim for a total dissolved solids concentration somewhere in the range of 25 to 150 mg/mL.

The quantity of drug, along with its solubility, determines the feasibility of administering the required dose in the appropriate volume of solution, and the required volume is determined by the intended route of injection. For continuous IV administration, there is no upper volume limit as long as the volumetric rate of infusion does not exceed the ability of the kidneys to eliminate the excess volume of water. For IV bolus administration, the injected volume is generally 10 mL or less. For intramuscular administration, up to about 5 mL is injected and for subcutaneous administration, the injected volume is up to about 1.5 mL. Of course, many drugs are weak acids and bases, where the solubility (and often solution stability) is strongly influenced by pH, so both a solubility versus pH profile and a stability versus pH profile are needed over a reasonable formulation pH range.

Some information is necessary on both the routes, and the rates, of chemical degradation in solution. If this were not an issue, then there would be no need for freeze-drying. If the drug degrades too rapidly in solution, then degradation during compounding, sterile filtration, filling, and transfer to a freeze dryer can present a significant challenge. For protein therapeutic agents, physical stability must be examined in solution as well, where physical stability generally refers to the tendency of proteins to form aggregates, either soluble or insoluble. This can occur either spontaneously in solution or as a result of denaturation in response to adsorption to solid surfaces such as tubing or filters or adsorption to the air-water interface present during processing.

EXCIPIENTS IN FREEZE-DRIED PRODUCTS

No formulation scientist wants to risk delay of an NDA submission by using unprecedented excipients, so the choices of excipients, particularly relative to those available for solid oral dosage forms, is limited (1). Below is a brief survey.

Buffers

By far the most common buffer system in freeze-dried parenterals is sodium phosphate, since it is present physiologically and has a pK near the pH of normal plasma. A risk associated with freeze-drying of solutions containing sodium phosphate is pH shifts with freezing, discussed in the following text. Other buffer systems used in approved products include acetate, citrate, arginine, histidine, succinate, and Tris (tris-hydroxymethyl aminomethane).

There is no "rule" that buffers must be included in a formulation. If no buffer is needed, then it is appropriate not to use one.

Bulking Agents

Bulking agents, mentioned earlier, are needed when the drug quantity is insufficient to form a pharmaceutically acceptable freeze-dried solid, and the drug is dispersed in an inert matrix that has appropriate freeze-drying characteristics. Bulking agents fall into two general categories those that tend to crystallize from a frozen system and those that remain amorphous. The most common crystallizing excipients are mannitol and glycine. Polyethylene glycols, which are less common, also tend to crystallize from freezing solutions. Whether these excipients actually crystallize depends largely on their concentration relative to other formulation components and, to a lesser extent, on the thermal history of freezing. Crystallizing excipients

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have the advantage of allowing freeze-drying at relatively high product temperatures (see discussion later in this chapter), which provides more efficient processing.

Mannitol is known to, in some cases, promote vial breakage during freeze-drying (2). In addition to causing loss of yield of acceptable product, this can create problems with containment of cytotoxic compounds. The detailed mechanism by which this happens is not well understood, but important contributing causes include the relative concentration of mannitol in the formulation and the relative fill depth. Rates of breakage increase significantly when the relative fill volume exceeds about one-third of the capacity of the vial. Thermal history of freezing has been shown to influence vial breakage as well. The vial specifications may also play a role particularly the heel radius, where the side wall joins with the bottom of the vial. The smaller the heel radius, the more the stress associated with expansion of the frozen system during freezing is concentrated, which in turn promotes vial breakage.

Amorphous excipients include disaccharides such as sucrose, trehalose, and, occasionally, lactose or maltose. These excipients may play a double role in a formulation both as a bulking agent and, for proteins and other biologicals, as a stabilizer. To be effective, the stabilizer must be amorphous and remain so throughout the shelf life of the product. Human serum albumin is used in several protein formulations both as a stabilizer of the protein in the solid state and as a competitive surfactant to inhibit loss of the active protein by adsorption to surfaces. A detailed discussion of mechanisms of stabilization of biological formulations is outside the scope of this chapter, and the reader is referred to publications by Carpenter et al. (3), Arakawa et al. (4), Cleland et al. (5), and Chang et al. (6).

Added Salts

Salts such a sodium chloride are often included in freeze-dried formulations to provide an isotonic reconstituted solution. Their use should be approached with some caution, however, for several reasons. First, when used in combination with amorphous excipients, added salt tends to decrease the collapse temperature (discussed later), making the process less efficient and, in some cases, increasing the risk of not being able to make a pharmaceutically acceptable product (7). Added salt may also inhibit crystallization of components of the formulation for which crystallization is needed. When added salt is needed in a formulation, it is particularly important to systematically vary the amount of salt and study the effect of salt concentration on the freeze-drying characteristics of the formulation.

THE FREEZING PROCESS

Freezing is a critical step in the freeze-drying process, since the physical state of the frozen system influences quality attributes of the final product as well as the process efficiency. Characterization of freezing behavior is an important step in the development of a freeze-dried product for several reasons. First, the driving force for freeze-drying is the vapor pressure of ice, and the vapor pressure of ice is very temperature dependent. A goal of process optimization is to carry out freeze-drying at the highest rate possible without causing damage to the product. Every frozen formulation has an upper temperature limit (more about this below) during the primary drying process, and it is important to know this upper temperature limit and use it in process development so that the product temperature remains safely below this limit during primary drying, but not so far below the limit as to make the process unnecessarily time consuming. Second, process validation involves assuring that the cycle conditions are appropriate for the formulation. To be able to validate a process, "benchmark" data must be available to assess the adequacy of the process conditions, and these data are provided by characterization of the formulation. Finally, there is ongoing regulatory scrutiny of the pharmaceutical development process as well as manufacturing operations. Regulatory authorities expect a scientific rationale for freeze dry cycle conditions, with appropriate documentation.

Types of Freezing Behavior

An overview of the events that can take place during freezing is shown by the diagram in Figure 4. For simplicity sake, it is assumed that the starting solution is a simple aqueous system. It is important to recognize that even pure water does not freeze at 0°C, but instead

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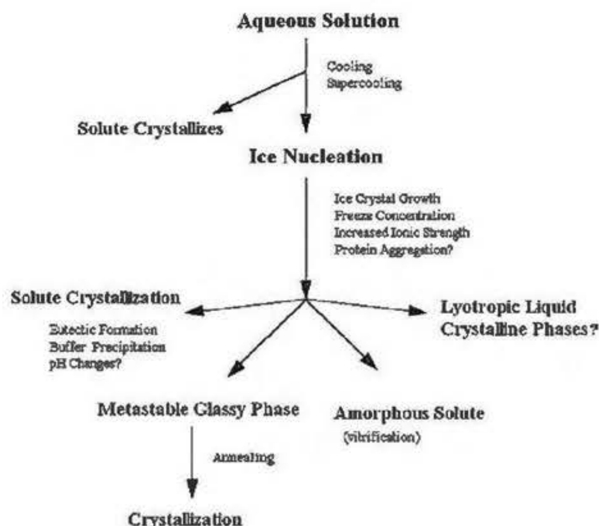

Figure 4 Schematic diagram of events taking place during freezing.

undergoes *supercooling*, where the water remains liquid well below the melting point of ice. Supercooling is important because the actual *freezing rate* of a solution is determined by the time elapsed between nucleation of the first ice crystals and complete solidification of the system. This is sometimes confused with the *cooling rate*, which is the rate at which the temperature of the shelves is decreased during freezing.

Ice nucleation, like any crystallization process, can be either *homogeneous*, where water molecules spontaneously order themselves into nuclei, or *heterogeneous*, where nucleation is triggered by a surface or by extraneous particulate matter. In practice, nucleation is always heterogeneous. The solutions we deal with in freeze-drying have been sterile filtered, and the containers (usually glass vials) have been cleaned and sterilized, so there is relatively little in the way of extraneous particulate matter to trigger ice nucleation. Nevertheless, there are still irregularities in the microstructure of the glass that can serve as nucleation sites, but aqueous solutions intended to be freeze-dried can supercool by as much as 12 to 15°C before ice crystals nucleate. High degrees of supercooling result in rapid freezing, which in turn influences ice crystal morphology and the amount of water in the system that remains unfrozen.

It is important to first consider ice morphology before considering the behavior of components of a formulation during freezing. Different ice morphology, including regular and irregular dendrites, as well as spherulitic systems (thin fibers if ice radiating outward from the nucleation site), forms during freezing, depending on the freezing rate and the type and concentration of solutes present. Ice crystal morphology and size distribution have been shown to influence the rates of primary (8) and secondary drying, as well as protein aggregation in freeze-dried protein formulations. Searles et al. (8) describe three stages of the freezing process. The first is the heterogeneous nucleation of ice or primary nucleation. This is followed by secondary nucleation, during which a visible front propagates through some portion of the sample at a rate on the order of several millimeters per second. This process stops as the temperature of the system approaches the equilibrium freezing temperature. Secondary nucleation is followed by solidification, which takes place at a slower rate as the heat released by ice crystallization is conducted out of the sample and ultimately to the heat transfer fluid. These investigators further describe two different freezing mechanisms. In the first, termed *global supercooling*, the entire liquid volume reaches the same degree of supercooling and the secondary nucleation zone includes the entire solution volume. In *directional solidification*, a portion of the liquid volume is cooled to the point of primary and secondary nucleation, and the nucleation and solidification fronts move together into the previously unnucleated portion of the solution. Both mechanisms can apply to the type of

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freezing that takes place in pharmaceutical freeze-drying; that is, vials filled with liquid that are frozen on the freeze dryer shelf. However, directional solidification usually requires some type of ice nucleating agent. The freezing mechanism was demonstrated to be reflected in the morphology of the freeze-dried cake.

Most people do not think of freezing as a dehydration process, but it certainly is in the sense that, when the water freezes, it becomes a separate phase, and the material in the interstitial space between ice crystals becomes much more concentrated. If a solution of normal saline is frozen for example, the sodium chloride concentration in the initial solution is 0.15 N. When this solution is frozen, the sodium chloride concentration in the interstitial space between ice crystals reaches nearly 4 N before sodium chloride precipitates from the freeze concentrate. In the case of formulations containing sodium chloride or other salts, this high ionic strength environment can be damaging, particularly to biological materials such as proteins and cells.

A main point of Figure 4 is that both freeze-drying behavior and quality attributes of the product are determined by the physical state of the solute, or solutes, in the frozen system. For the sake of simplicity, we will assume a single solute dissolved in water. After ice crystal growth has essentially completed, and the solute has been concentrated as much as possible in the interstitial space between ice crystals, what happens next depends on whether the solute crystallizes from this freeze concentrate.

Solute Crystallizes from the Freeze Concentrate

When the solute crystallizes from the freeze-concentrated solution, the phase behavior is represented by a temperature-composition phase diagram as shown in Figure 5. In a simple solution, 100% A represents pure water and 100% B represents pure solute. We will use sodium chloride as B in this example. The curve AC represents the melting point of ice as a function of sodium chloride concentration, and the curve BC represents the solubility of sodium chloride in water as a function of temperature. If the system is cooled to a temperature below point C, the solute is no longer soluble, and it crystallizes and precipitates. This is the *eutectic* (from Greek, meaning "easily melted") *composition*, and the temperature corresponding to C is the *eutectic melting* temperature.

What the phase diagram tells us about freeze-drying of a solution of sodium chloride in water is the following. Starting with a solution of sodium chloride in water, say at point x in Figure 5, and cooling this solution to perhaps 40°C, we follow a vertical line on the phase diagram. Below the freezing point curve, we have a two-phase system ice and a freeze-concentrated solution of sodium chloride. In the two-phase region, as the temperature is

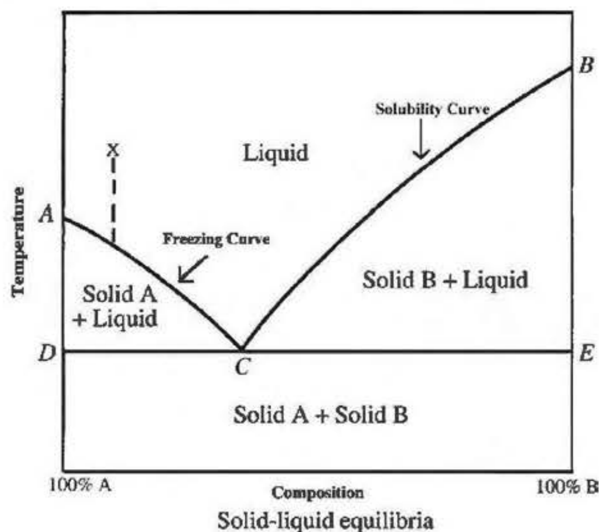

Figure 5 Temperature composition phase diagram of a binary system.

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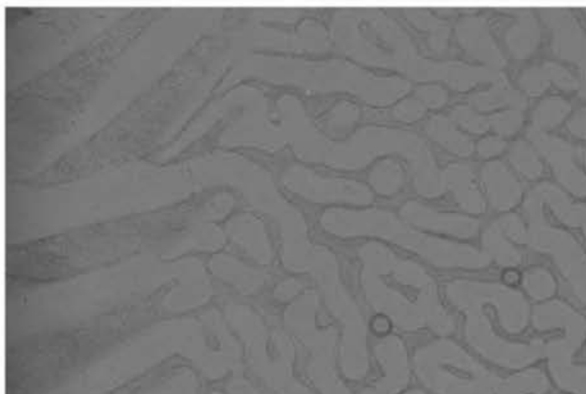

Figure 6 (*See color insert*) Photomicrograph of a frozen solution of sodium chloride in water.

decreased, the ice crystals grow and the freeze concentrate becomes more concentrated. At any temperature in this region, the composition of the system is given by a horizontal line (called a "tie line") through this temperature. Thus, the composition of the freeze concentrate is given by the line AC. When this concentration reaches point C, a eutectic mixture of sodium chloride and ice precipitates form the freeze concentrate. It is only at this point that the system is completely solidified. A photomicrograph of a frozen sodium chloride solution is shown in Figure 6, where the dark material in the interstitial space between ice crystals is the eutectic mixture. This mixture consists of ice and crystalline sodium chloride. The reason that these crystals cannot be seen in the photograph is that the crystallite size is too small to be resolved by an optical microscope.

In reality, the crystallization of solute is just as unpredictable as the crystallization of water. While it cannot be shown on an equilibrium phase diagram, supercooling in these systems occurs twice once prior to crystallization of ice, and again prior to crystallization of the eutectic mixture. Both events require nucleation, which is a stochastic process.

Eutectic mixtures melt at a sharply defined temperature, as if they were a single, pure compound. The significance of the eutectic melting temperature to freeze-drying is that it represents the maximum allowable product temperature during primary drying. Exceeding this temperature during the process would result in puffing, foaming, perhaps expulsion of solids from a vial, and loss of pharmaceutical acceptability. A list of eutectic melting temperatures of some pharmaceutically relevant materials is shown in Table 2. However, despite the attention that eutectic solidification has attracted in the literature and the considerations given to it in this text, it is not seen with most formulations. The most common behavior is formation of a glassy mixture, discussed in the following text.

Eutectic crystallization is the underlying cause of a phenomenon in freezing of formulations intended for freeze-drying that is worthy of mention. Solutions of sodium phosphate, the most common buffer in freeze-dried formulations, are known to undergo a pH shift accompanying freezing, such that the effective pH in the freeze concentrate formed

Table 2 Eutectic Melting Temperatures of Representative Materials

Material	Eutectic melting temperature (°C)
Mannitol	About 1
Glycine	3.5
Sodium phosphate dibasic	0.5
Polyethylene glycols	14 to 16
Sodium chloride	21.5
Citric acid	12
Potassium chloride	10.7
Sodium acetate	18

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during freezing can be significantly lower than that of the starting solution. This happens because the dibasic buffer salt is less soluble at low temperature than the monobasic salt. Crystallization of the dibasic sodium phosphate causes the equilibrium to shift according to LeChatlier's Principle, resulting in decreased pH. Gomez and Rodriguez-Hornedo (9) used a special pH electrode designed to withstand freezing to study the influences of initial buffer solution pH and concentration on subsequent pH changes during freezing, as well as the influence of other species in solution on buffer salt crystallization. These investigators reported that the pH changes associated with crystallization of a sodium phosphate buffer solution initially at pH 7.4 are directly related to the initial concentration of buffer in the range of 8 to 100 mM. Further, the lower the initial pH of the buffer, the higher the observed pH at 10°C. Addition of NaCl increases the ion product of dibasic sodium phosphate, thereby leading to larger pH changes. Solutes such as sucrose and mannitol inhibited crystallization of buffer species, resulting in smaller pH shifts upon freezing. The presence of sucrose and mannitol at concentrations above 3 moles per mole of dibasic sodium phosphate completely prevented buffer salt crystallization. In this case, the pH change upon freezing was only 0.5 units, which was attributed to the effect of freeze concentration. It is worth emphasizing that pH shifts only occur when the dibasic sodium phosphate salt crystallizes, so just because a formulation contains sodium phosphate does not mean that the pH will shift during freezing. Other components of formulation, particularly those that remain amorphous during and after freezing, as well as rapid freezing rates, tend to inhibit this crystallization.

Other pharmaceutically relevant buffer systems have not been as well characterized as phosphate with respect to pH changes accompanying freezing. Larsen (10) reported that acetate, citrate, glycine, and Tris show only small pH shifts upon freezing.

Many drugs crystallize readily from freezing aqueous solutions, including sodium ethacrynate, pentamidine isethionate, nicotinamide, ribavirin. Common excipients that readily crystallize, in addition to sodium chloride and sodium phosphate dibasic, are mannitol, glycine, and polyethylene glycols.

Solute Remains Amorphous After Freeze Concentration

Again, to simplify the discussion, we are considering aqueous systems containing only one solute. Consider sucrose, a common excipient in freeze-dried protein formulations. In this case, the first part of the process is the same; that is, ice crystals nucleate after considerable supercooling and grow. The freeze concentrate becomes more concentrated in sucrose and more viscous. However, unlike sodium chloride, sucrose does not crystallize from the freeze concentrate regardless of the freezing temperature used, and its behavior cannot be described by an equilibrium phase diagram. The glassy mixture resulting from freeze concentration does undergo a *glass transition* as the temperature decreases, where the viscosity of the mixture may increase by orders of magnitude over a temperature range of a few degrees. This glass transition is a reversible change of state (it is not a phase change) between a viscous liquid above the glass transition to a solid below the glass transition. The glass transition temperature of the maximally freeze-concentrated solute, known as T_g', is the physical chemical basis for *collapse* in freeze-drying. If the temperature of the product is held below T_g', the glassy mixture of solute and unfrozen water is rigid enough to support its own weight as the supporting structure of ice crystals is sublimed away. This results in retention of the microstructure that was established by the freezing process. If, however, the temperature of the system is increased above T_g' during primary drying, the glassy mixture of solute and water can undergo viscous flow under the force of gravity when ice is sublimed, a phenomenon known as collapse.

A pharmaceutically acceptable freeze-dried solid generally has the same size and shape as the liquid that was originally filled into the vials, and has a uniform color and texture. These qualities are generally lost as a result of collapse. In addition, collapse results in a decrease in the specific surface area of the freeze-dried solids, and this can result in longer reconstitution time relative to a system that retains the microstructure established by freezing. Perhaps more importantly, collapsed systems tend to have higher levels of residual moisture, perhaps because of decreased surface area available for evaporation of the water that was part of the glassy mixture. This, in turn, can adversely influence stability of the freeze-dried solid. A

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Figure 7 The vial on the left shows complete collapse and the second vial from the left is an example of partial collapse. The two vials on the right are pharmaceutically acceptable.

photograph of a vial exhibiting collapse is shown in Figure 7, and a list of T_g' temperatures of representative materials is given in Table 3. It is important to recognize that both T_g' and collapse temperatures are more subjective measurements than, for example, melting temperatures. This is discussed further in the section later on dealing with characterization of frozen systems.

Figure 8 is a cartoon intended to illustrate the concepts discussed above. There are important differences in freeze-drying behavior between systems where the solute crystallizes and those where it remains amorphous. First, when the solute crystallizes, nearly all of the water in the system is frozen, either as pre-eutectic ice or ice that is part of the eutectic mixture. This means that there is very little secondary drying required. Amorphous systems, on the other hand, contain a significant amount of unfrozen water. Maximally freeze-concentrated sucrose, for example, contains about 20% unfrozen water, which requires removal during secondary drying. Second, eutectic melting temperatures of most pharmaceutically relevant materials tend to be fairly high in the range of 1°C to about 15°C. Glass transition temperatures, on the other hand, vary over a much wider range (Table 3), and can be so low that the system cannot be completely solidified in a freeze dryer, where the lowest temperature achieved on the shelf is seldom below about 45°C.

In addition to different behavior during freeze-drying, the physical state of the drug can dramatically influence the stability of the freeze-dried solid. Amorphous drugs can undergo solid-state degradation at substantially higher rates than the same drug as a crystalline solid (11).

Of course, actual formulations usually consist of several components, so it is not uncommon to see both types of behavior within the same formulation, where there is a crystalline component and an amorphous component. In this case, the freeze dry cycle conditions must be based on the lowest of either the eutectic melting temperature or the collapse temperature. This is usually the collapse temperature.

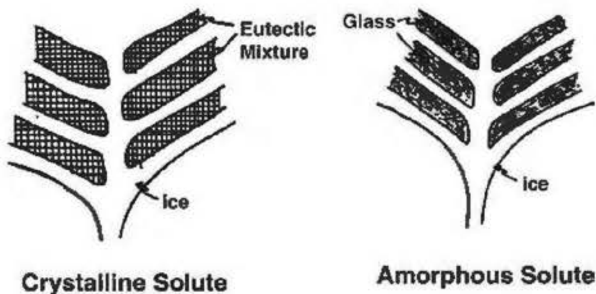

Figure 8 Cartoon showing the micro structure of a frozen systems in which the solute is crystalline and amorphous.

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Table 3 T_g' Temperatures of Representative Materials

Material	T_g'
Dextran	9°
Fructose	48°
Gelatin	8 to 10°C
Sucrose	32° to 34°
Lactose	32°
Maltose	32°C
Trehalose	30°
Albumin	10°
Glycine (amorphous)	< 60°

Solute Forms a Metastable Glass

Sometimes, perhaps because of a high degree of supercooling and a subsequent rapid freezing rate, a compound will first form a glassy mixture in the interstitial space between ice crystals; however, with some subsequent heating, the solute will crystallize from this glassy mixture. Mannitol is the most common example of metastable glass formation. For this reason, *annealing* is sometimes used in a freeze-drying process. Annealing is simply heating the "frozen" system after the initial freezing process not enough to melt the product, but enough to promote crystallization of components of the formulation that have initially formed glassy mixtures. Typically, an annealing step would consist of heating the frozen system to a temperature higher than T_g' but lower than the onset of melting, and holding for two to three hours. Gatlin and DeLuca (12) investigated three cephalosporins that all form glassy mixtures upon initial freezing and, unless annealed, remained in the less desirable amorphous form after freeze-drying.

Solute Forms a Lyotropic Liquid Crystal

States of matter which have degrees of order intermediate between amorphous and crystalline are called liquid crystals. Liquid crystals are broadly categorized as *thermotropic*, which are formed by heating, and *lyotropic*, which are formed by addition of solvent to a solid. Compounds that form liquid crystals are generally surface active, and the liquid crystal represents a more ordered structure than a micelle. These higher-ordered structures are a result of freeze concentration and may be either lamellar or rod shaped. There have been few reports of lyotropic liquid crystal formation in aqueous solutions of drugs, and even fewer that are relevant to freeze-drying. Powell and co-workers (13) reported peptide liquid crystal formation by the luteinizing hormone releasing hormone deterelix and the effect of added salts on thermodynamic stability of the liquid crystal phase. Vadas et al. (14) reported that a leukotriene D_4 receptor antagonist forms lyotropic liquid crystalline phases when lyophilized from aqueous solution. Bogardus (15) studied the phase equilibria of nafcillin sodium-water and reported a lamellar mesophase in aqueous solutions containing more than 55% nafcillin sodium. Milton and Nail (16) extended this work by characterizing the low-temperature differential scanning calorimetry (DSC) thermogram of frozen aqueous solutions of nafcillin as well as the freeze-dried solid. Freeze-drying of frozen systems containing lyotropic mesophases appears to result in a unique x-ray diffractogram consisting of a single sharp peak at low angle (less than about 5° 2θ) in addition to the "halo" that is characteristic of amorphous solids. Herman et al. (17) reported a similar x-ray powder diffraction pattern in methylprednisolone sodium succinate. The influence of liquid crystal formation during freezing on critical quality attributes of freeze-dried products is a subject that remains largely unexplored.

Characterization of Freezing Behavior

The purpose of characterizing the freezing behavior of a formulation intended for freeze-drying is primarily to determine the maximum allowable product temperature during the primary drying phase as well as to gain insight into the physical state of the material during

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and after freeze-drying. While a variety of methods have been reported in the literature, we will briefly describe the two most common characterization methods low-temperature thermal analysis and freeze dry microscopy.

Thermal Analysis

Thermal analysis of frozen systems intended for freeze-drying has become a standard tool for formulation and process development (18 20). Physical or chemical changes in a material occurring with changes in temperature are accompanied by the absorption or release of energy in the form of heat. Thermal analysis measures the temperatures at which these transitions occur, as well as the energy associated with the transitions and whether they are endothermic (absorption of energy) or exothermic (release of energy). The types of transitions observed in frozen formulations are illustrated in Figure 9. These are only three. The glass transition of the maximally freeze-concentrated solute is observed as a shift in the baseline toward higher heat capacity. Eutectic melting is an endothermic peak, and crystallization of a formulation component during the time course of the DSC experiment is observed as an exotherm. Thus, interpretation of DSC thermograms of frozen systems is simple in principle. However, several factors contribute to uncertainty in interpretation of the DSC data. First, eutectic melting may take place at a temperature very near the melting endotherm of ice; for example, eutectic mixtures of mannitol/ice and dibasic sodium phosphate/ice undergo eutectic melting at about 1 and 0.5°C, respectively. These endotherms are not resolved from the melting of ice. Therefore, resolution of eutectic melting from ice melting can be a significant source of uncertainty. Regarding the glass transition, in some cases the heat capacity change is too small to be detected by DSC with certainty, so sensitivity can become an issue. It should also be noted that glasses are broadly defined as *strong* or *fragile*. This does not refer to mechanical properties directly, but rather to the temperature dependence of molecular mobility in the region of the glass transition. Fragile glasses have relatively narrow glass transition regions and relatively high heat capacity change associated with the glass transition. Strong glasses are the opposite they have broad glass transition regions and small heat capacity change associated with this transition. Therefore, the glass transition can be difficult to detect for strong glasses. Fortunately, most pharmaceutically relevant amorphous materials (with the exception of proteins) are fragile glasses. Interpretation of low-temperature DSC thermograms can also be more uncertain in formulations containing many components, probably because of interactions between components. Finally, the glass transition region, particularly for systems containing more than about 10% of an amorphous solute, may be observed as more than a

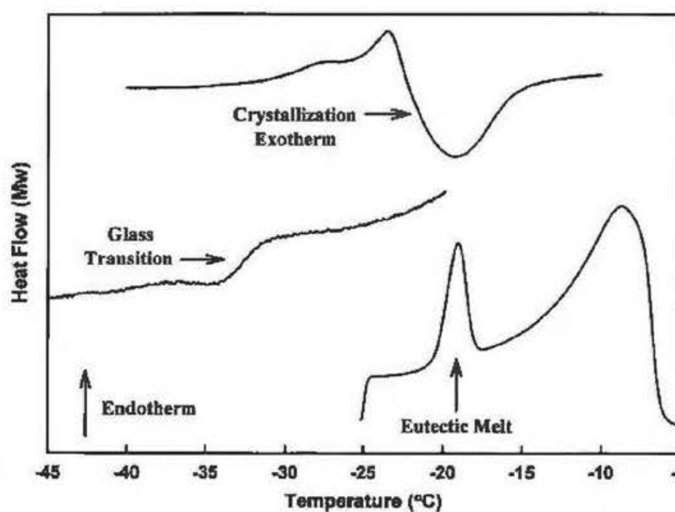

Figure 9 Thermal transitions occurring in frozen systems.

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single transition. Sacha and co-workers (21) have shown that disaccharides share this "double transition" feature, and that the higher-temperature transition is the transition that is most predictive of collapse in freeze-drying.

In the past 15 years or so, *modulated* DSC has become a common tool for characterization of frozen systems. In this method, the temperature is changed linearly with superposed sinusoidal temperature modulation, and the sample thermal response is observed in comparison with that of the thermally inert reference material. The sample thermal response is separated by Fourier transformation into a response in-phase with the temperature modulation and a response that is out of phase with the modulation. The response that is in-phase is recorded as the reversing component of the thermogram, and the out-of-phase response is recorded as the nonreversing component. Modulated DSC helps to resolve different thermal events occurring in the same temperature range; for example, a crystallization exotherm could tend to obscure a glass transition, and modulated DSC separates these events into the two components of the thermogram. Modulated DSC is useful both in characterization of frozen systems and in characterization of freeze-dried solids particularly amorphous solids. A detailed discussion of modulated DSC is beyond the scope of this chapter. The reader is referred to reviews by Coleman and Craig (22), Schawe (23), and Ozawa (24).

Freeze Dry Microscopy

Freeze dry microscopy refers to observation of freezing and freeze-drying behavior using a freeze-drying stage mounted on an optical microscope. Such stages were, in earlier days, homemade devices but are now available commercially. The stage shown in Figure 10 consists of a metal block with a hole to allow the sample to be illuminated with transmitted light. The temperature of this block is controlled by a combination of an electrical heater embedded in the block and the circulation of the nitrogen that boils off a Dewar containing liquid nitrogen. A sample volume of no more than about 5 μL is placed on a microscope cover slip, which is placed on the block, and another cover slip is placed on top. It is good practice to use a small drop of a coupling fluid such as silicone oil to assure good thermal contact between the metal block and the bottom cover slip. There is a removable lid on the stage with a window for viewing. The stage is connected to a vacuum pump and to a pressure gauge. The experiment then consists of freezing the sample, evacuating the stage, and carrying out primary drying. Sample temperatures and ramp rates can generally be programmed.

It is helpful to use a microscopy with polarizing capability to gain information about the physical state of the sample. Crystalline materials, generally having more than one refractive index, give rise to retardation colors, whereas amorphous materials are dark under normal conditions of illumination. The working distance of the microscope the distance between the

Figure 10 Stage for freeze dry microscopy.

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sample and the tip of the ocular should be about 1 cm to allow space for the lid on the top of the stage.

During primary drying, a distinct sublimation front can be observed moving through the frozen material. At the onset of collapse for amorphous systems, viscous flow of the freeze-concentrated material can be observed as the supporting structure of ice crystals sublimes away. This is illustrated by the photomicrograph in Figure 11. While the underlying event behind collapse of the sample is the glass transition of the freeze-concentrated material, collapse of the sample is not always observed at the same temperature as T_g'. The reason for this is that even though primary drying and secondary drying are different phases of the drying process, once the sublimation front passes through a given volume element of a sample, secondary drying of the partially dried material in that volume element begins, even though there is still ice in other regions of the sample. As secondary drying proceeds, unfrozen water is removed, which deplasticizes the material, causing the glass transition temperature of the partially dried material to increase. For this reason, collapse is generally observed at a somewhat higher temperature than T_g'. How much higher depends on both the rate of primary drying and the rate of secondary drying, but about 3°C is representative.

Collapse is caused by viscous flow of the partially dried material when ice is removed, so it is observed in the dried layer, usually immediately adjacent to the sublimation front (because it is in this region that the level of unfrozen water is highest). Eutectic melting, on the other hand, is observed in the frozen layer, and is usually accompanied by bubbling of the sample as air bubbles formed during freezing expand into the vacuum.

Freeze dry microscopy is also useful for observing annealing effects in freeze-drying. As the frozen material is annealed, the morphology of the ice is likely to change as ice crystals grow. Annealing also may promote crystallization of materials that remain amorphous initially after freezing. Figure 12 illustrates the crystallization of nafcillin during annealing of the frozen solution. The circles represent areas where crystallization has taken place.

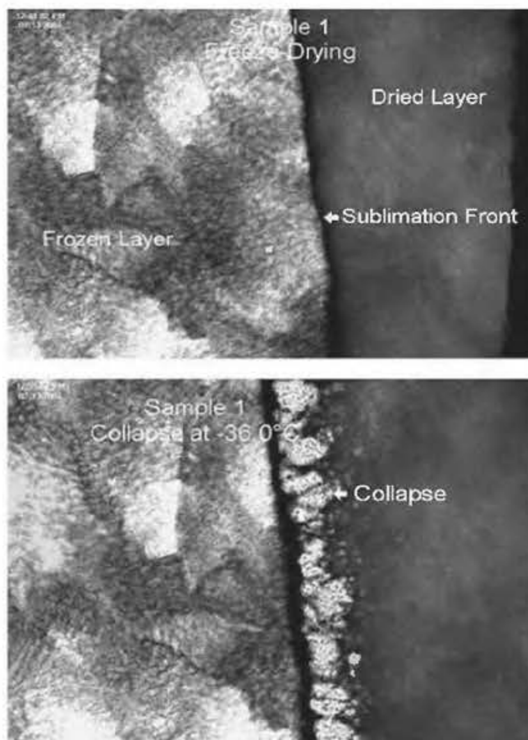

Figure 11 (*See color insert*) Photomicro graphs taken during freeze drying showing reten tion of structure (*top*) and collapse (adjacent to sublimation front).

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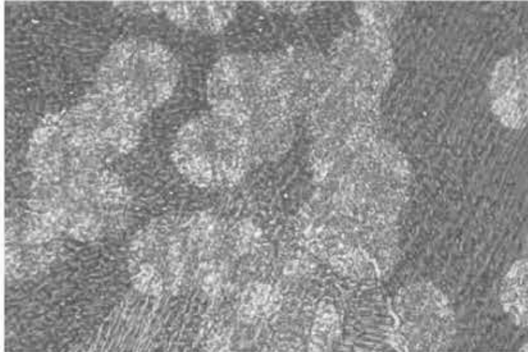

Figure 12 (*See color insert*) Photomicrograph showing crystallization of a solute from a frozen system during annealing.

Perhaps the most important source of uncertainty in the use of a freeze-drying microscope is the uncertainty around determination of a collapse temperature. Some formulations undergo collapse rather abruptly as the temperature of the system is increased. Others, however, collapse very gradually perhaps over a range of 10°C or more. The most relevant information is the level of collapse that can be detected visually in the freeze-dried solids. However, the level of microscopic collapse that would correspond to visually observable collapse is not obvious.

HEAT AND MASS TRANSFER OPERATIONS IN FREEZE-DRYING

Sublimation of ice is not inherently slow. The maximum rate of evaporation (or sublimation) is a function of the vapor pressure of the substance at a given temperature, as well as the molecular weight of the material. Using appropriate values for water, and assuming 2 mL of water in a vial, leads to the conclusion that if the ice were allowed to sublime at its maximum rate, the ice would be removed in about one minute. Why, then, does it take two days or more to freeze dry many actual formulations? The answer is because of limitations in the rate at which the heat of sublimation can be provided where it is needed, and the rate at which water vapor can be transported from the sublimation front and deposited on the condenser.

Heat and mass transfer in freeze-drying are related through the heat of sublimation of ice, ΔH_s, which is about 2828 J/g. Heat and mass transfer, like all transfer operations, follow the general form of

$$\text{Flux (or flow rate)} = \text{Driving force} \times \text{Conductance}$$

Alternatively, the flow term can be expressed as a driving force divided by a resistance. For heat transfer, the flow rate term refers to the rate of heat transfer, the driving force is a temperature difference, and the conductance term might be the thermal conductivity of a material, such as ice. For mass transfer, the flow rate term is the sublimation rate, the driving force is a pressure difference, and conductance term is generally expressed as a resistance to mass transfer. Complications arise, though, because freeze-drying takes place in a system at low pressure, and the transport properties of the vapor are a function of the system pressure. Before proceeding further, it is helpful to briefly discuss the transport properties of gases at low pressure, since this is important to understanding both heat transfer and mass transfer in freeze-drying.

Transport Properties of Gases at Low Pressure

A fundamental property of gases at low pressure as regards transport properties of gases is the *Knudsen number*, or *Kn*, which is the ratio of the *molecular mean free path*, *L*, to a characteristic dimension of the system, *a*. For water vapor, the mean free path is roughly

$$L(\text{cm}) = \frac{3}{P_\mu}$$

where the pressure, *P*, is expressed in microns of mercury (μHg) or millitorr (mT). For example, at 50 μHg, which is representative of pressures used in freeze-drying, the mean free

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path of water vapor is about 0.06 cm. The characteristic dimension of the system depends on what aspect of freeze-drying we are addressing. For example, if the issue is flow of vapor through the pores of the partially dried solids, a is the average diameter of the pores in the cake. For heat transfer from the shelf to the vial, we must take into account the fact that the bottom of the vial is not flat, and is therefore not in intimate contact with the shelf. In this case, the value of a is the average thickness of the "gap" between the bottom of the vial and the shelf. If the issue is flow of vapor through the duct connecting the chamber of the freeze dryer with the condenser, a would be the diameter of the duct.

When the mean free path is small compared to the characteristic dimension a, collisions between gas molecules are much more frequent than collisions with the boundaries of the system under consideration. Since collisions between molecules determine the viscosity of the gas, this flow regime is called *viscous flow*. If L is large compared to a, then collisions of molecules with the boundaries of the system dominate, and the flow is termed *free molecular*, or *Knudsen*, flow. A third flow regime is *transition* flow, which lies between viscous and Knudsen flow. Approximate values of Kn delineating the different flow regimes are:

$$
\begin{aligned}
Kn &< 0.01 && \text{viscous flow}\\
0.01 < Kn &< 1 && \text{transition flow}\\
1 &< Kn && \text{free molecular flow}
\end{aligned}
$$

Flow regime has a dramatic effect on transport properties. This will be discussed separately for heat transfer and mass transfer.

Heat Transfer at Low Pressures

Under viscous flow conditions, the conductance of heat through a gas is independent of pressure, and Fourier's law applies:

$$\frac{dQ}{dt} = kA \times \frac{dT}{dx}$$

where dQ/dt represents the rate of heat transfer; k is the *thermal conductivity* of a material; A, the area at right angles to the direction of heat flow; and dT/dx is the temperature gradient (the driving force). For water vapor, the thermal conductivity is about 0.64 J/hr cm °K at 273°K.

Heat transfer under free molecular flow conditions is more complicated. Knudsen developed the theory of thermal conductance of gases in this flow regime based on collisions of individual molecules with a surface. As discussed earlier, the gas behavior in this range is determined by collisions of molecules with the boundaries of a system, not on collisions between gas molecules. When a molecule at a temperature T_i strikes a surface at a higher temperature T_s, the incident molecule picks up thermal energy from the collision. The extent to which the energy is increased by the collision is expressed by a term known as the *accommodation coefficient*, α, where

$$\alpha = \frac{T_r \quad T_i}{T_s \quad T_i}$$

and T_r is the temperature of the gas molecule rebounding from the collision with the warmer surface. If $\alpha = 1$, then the exchange of energy is complete, and the molecule acquires the temperature of the warmer surface after one collision. Using the kinetic theory of gases, it can be shown that the rate of energy transfer from a hot surface to a cold surface per unit area is

$$E_0 = \alpha \Lambda_0 P (273/T_i)^{1/2} (T_s \quad T_i)$$

Note that, in the free molecular flow regime, the rate of energy transfer is directly related to the system pressure. The quantity Λ_0 is the *free molecular heat conductivity* at 0°C. Table 4 gives some values of Λ_0 for representative gases. Note that the conductance of water vapor of water vapor is significantly higher than that of air. Note also that the rate of energy transfer is *independent* of the distance separating the bodies exchanging heat. This makes sense in light of

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Table 4 Values of Free Molecular Heat Conductivity for Representative Gases

Gas	Free molecular heat conductivity (J/cm^2 hr °Kμ)
Hydrogen	$21.8 \times 10^{\ 2}$
Helium	$10.5 \times 10^{\ 2}$
Water vapor	$9.54 \times 10^{\ 2}$
Nitrogen	$5.76 \times 10^{\ 2}$
Oxygen	$5.58 \times 10^{\ 2}$

Source: From Ref. 25.

the fact that the behavior of the gas is dependent on collisions of gas molecules with the surfaces, not with other gas molecules.

Consider, as an example, steady-state heat transfer between two parallel plates separated by a distance x of 0.1 cm, with one plate at a temperature of 0°C and the other at 20°C. One millimeter would be roughly the effective separation distance resulting from the bottom of glass vials not being flat. First consider a system under vacuum containing only water vapor at a pressure of 1000 mT, where the molecular mean free path is 0.003 cm, and viscous flow conditions apply. Fourier's law applies and, assuming that k does not change much with temperature and the areas are constant,

$$\begin{aligned} \frac{Q}{At} &= \frac{k\Delta T}{x} \\ &= (0.64\ \text{J/cm hr}\ ^\circ\text{K})(20^\circ\text{K})/0.1\ \text{cm} \\ &= 128\ \text{J/cm}^2\ \text{hr} \end{aligned}$$

Now consider the same system, but at a pressure of 1 μHg (1 mT), where the mean free path is about 3 cm and free molecular flow conditions apply. Assuming an energy accommodation coefficient value of 0.9 and a water vapor temperature of 0°C,

$$\begin{aligned} E_0 &= (0.9)(9.54 \times 10^{-2}\ \text{J/hr cm}^2\ ^\circ\text{K mT})(1\ \text{mT})(20^\circ\text{C}) \\ &= 1.71\ \text{J/hr cm}^2 \end{aligned}$$

Thus, for the same driving force (the temperature difference between the two surfaces), evacuating the system to a pressure in the free molecular flow regime decreases the rate of heat transfer by about a factor of roughly 75. Of course, actual freeze-drying takes place at pressures intermediate between these pressures, so the conductive heat transfer consists of components of both viscous flow and free molecular flow, but the viscous flow component would tend to dominate over the free molecular component.

Mass Transfer at Low Pressures

Consider the flow of gas through a tube for both viscous flow and free molecular flow. Viscous flow is described by the Poiseuille equation, where the flow of gas, Q, through a straight tube of constant circular cross section is

$$Q = \frac{\pi r^4 P_a (P_2 \quad P_1)}{8\eta l}$$

where r is the tube radius; l, the tube length; and η, is the viscosity of the gas. P_a is the average of the upstream and downstream pressures, P_2 and P_1, respectively. The conductance F of this tube is:

$$F = \frac{Q}{P_2 \quad P_1} = \frac{\pi r^4 P_a}{8\eta l}$$

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Note that the conductance increases with the fourth power of the radius, is directly proportional to the average pressure in the tube, and is inversely proportional to the viscosity of the vapor and the length of the tube.

For *molecular flow* through a similar tube of constant cross-sectional area A, perimeter H, and length l, the flow rate is:

$$Q = \frac{(4/3)v_a A^2 (P_2 \quad P_1)}{Hl}$$

where v_a is the mean molecular speed and is given by

$$v_a = \left(\frac{8R_0T}{\pi M}\right)^{1/2}$$

where R_0 is the gas constant (8.31×10^7 ergs/°K g mole) and M is the molecular weight of the gas. The conductance is then

$$F = \left(\frac{4}{3}\right)\left(\frac{A^2}{Hl}\right)\left(\frac{8R_0T}{\pi M}\right)^{1/2}$$

Note that, for molecular flow, the conductance of the tube is independent of pressure. This makes sense, since the flow properties are determined by collisions of gas molecules with the boundaries of the system, and not by collisions between gas molecules. For a given gas at a constant temperature, the conductance depends only on the geometry of the tube. Conductance increases with the square of the area and is inversely proportional to the length of the tube.

To illustrate the influence of flow regime on conductance of a tube, consider a cylindrical tube with a radius of 1 cm and a length of 100 cm. For water vapor at an average pressure of 1000 mT and a temperature of 20°C, the mean free path, L, is 0.003 cm, so viscous flow conditions apply. The conductance of this tube is

$$F = \left(\frac{\pi r^4}{8\eta l}\right)P_a$$

The viscosity of water vapor at 20°C is 1.55×10^{-4} poise. Therefore,

$$F = \frac{\pi(1\,\text{cm})^4(1000\,\text{mT})}{8(1.55 \times 10^{-4}\text{g/cm sec})(1\,\text{cm})} = 101.3\,\text{L/sec}$$

Now consider the same tube at a pressure of 1 μHg, where the mean free path is about 3 cm and molecular flow conditions apply.

$$\begin{aligned} F &= (4/3)(\text{A}^2/\text{HI})(8R_0T/\pi M)^{1/2} \\ &= (4/3)[9.68\,\text{cm}^4/(6.28\,\text{cm})(100\,\text{cm})][8(8.31 \times 10^7\,\text{ergs/°K g mole})(253\text{°K})/\pi(18\,\text{g})]^{1/2} \\ &= 1.14\,\text{L/sec} \end{aligned}$$

Note the two order of magnitude difference in conductance between viscous flow conditions and free molecular flow conditions for the same tube. These are approximations only. There are several assumptions in the use of the Poiseuille equation to describe conductance. The reader is referred to Dushman and Lafferty for a more detailed discussion (25).

Heat Transfer in Freeze-Drying

There are three basic mechanisms for heat transfer conduction, convection, and thermal radiation. Conduction is the transfer of heat by molecular motion between one volume element of a material and the next. Convection is the transfer of heat by flow of a fluid either a liquid or a gas. Convection can be either *natural* convection, where the flow arises from density changes with temperature, or *forced* convection, where an external force is applied. Thermal radiation is electromagnetic radiation arising from thermal excitation of materials. It is generally accepted that, because freeze-drying takes place at a fairly low pressure, on the order

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of 0.1 mmHg, convection plays little, if any, role in freeze-drying, and it will not be discussed further here.

As discussed earlier, heat transfer by conduction is governed by Fourier's law:

$$Q = k \cdot \Delta T/\Delta x$$

where Q is the heat flux, or the rate of heat flow per unit area per unit time; k, the thermal conductivity of a given material; ΔT is the temperature difference between the two bodies exchanging heat, and Δx is the thickness of the material. Conductive heat transfer often takes place through a series of different materials; for example, heat transfer from the freeze dryer shelf to a vial undergoing sublimation requires conductance from the glass vial, the frozen formulation, and perhaps a tray between the vials and the shelf. In this case, a resistance term is defined as

$$R_i = \frac{x_i}{k_i}$$

where x_i and k_i are the thickness and thermal conductivity, respectively, of a given material. Thermal conductivities of representative materials are given in Table 5. In heat transfer through a series of resistances, there is usually one resistance that dominates the others, called the *limiting resistance*. In the case of freeze-drying formulations in vials, the limiting resistance arises from the fact that the bottom of a vial is not flat, and not in intimate contact with the heat source. The heat transfer rate is thus governed by the gas phase between the shelf and the vial as discussed earlier. Before proceeding further, it is important to discuss another mechanism of heat transfer *thermal radiation*.

Heat Transfer by Thermal Radiation

Heat transfer by thermal radiation is fundamentally different from heat transfer by conduction or convection, since some form of matter between the heat source and the heat sink is required for convection or conduction, whereas any matter between heat source and heat sink only impedes heat transfer by radiation. When thermal radiation strikes a surface, it may be absorbed, reflected, or transmitted. For most solids, the transmissivity is essentially zero, since they are opaque to thermal radiation. A hypothetical material, called a *black body*, has an absorptivity value of 1 and neither transmits nor reflects thermal radiation. Instead, all incident energy is absorbed and re-radiated. Real materials do not absorb all incident radiation and are termed *gray*. The *emissivity*, ε, is defined as the ratio of the total emissive power of a surface to the total emissive power of an ideally radiating surface, or black body, at the same temperature. At thermal equilibrium, the absorptivity and emissivity of a material are equal. Emissivity values for materials common to freeze-drying are listed in Table 6. It is important to note that emissivity of a given material is determined not only by the nature of the material,

Table 5 Thermal Conductivities of Representative Materials

Material	Thermal conductivity (J/cm hr°K)
Borosilicate glass	39.3
Aluminum	1.08×10^4
Stainless steel, type 304	618.6
Ice	78.2
Air (atmospheric pressure)	0.87

Table 6 Thermal Emissivity of Representative Materials

Material	Emissivity
Stainless steel, smooth	0.64
Glass, smooth	0.94
Aluminum, polished	0.04

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but also by the surface finish. In general, the more "shiny" a surface, the lower the thermal emissivity.

The rate of heat transfer by thermal radiation is given by the Stefan Boltzmann law:

$$Q = \sigma \varepsilon T^4$$

where σ, the Stefan Boltzmann constant, has a value of 2.04×10^{-8} J/cm^2 hr °K^4 and T is the absolute temperature. The quantity of heat transferred by a black body at temperature T_1 to a black body at a lower temperature T_2 is given by

$$\frac{Q_{12}}{A} = \sigma F_{12}(T_1^4 \quad T_2^4)$$

where F_{12} is the "view factor", which represent the fraction of total radiation leaving body 1 that strikes body 2. For gray body radiation, the view factor takes into account the emissivities of the two bodies in addition to the system geometry:

$$\mathcal{F}_{12} = [(1/F_{12}) + (1/\varepsilon_1 \quad 1) + (A_1/A_2)(1/\varepsilon_2 \quad 1)]^{-1}$$

These relationships can be used to estimate the contribution of thermal radiation to freeze-drying. Consider radiative heat transfer between two parallel plates of equal area, one representing a stainless steel freeze dryer shelf at 0°C and the other a glass plate representing an array of vials at 20°C. The thermal emissivities of the stainless steel and glass are assumed to be 0.64 and 0.94, respectively. Further, we assume that all of the thermal radiation from the stainless steel plate strikes the glass surface. The view factor is then

$$F_{12} = [1 + (1/0.64 \quad 1) + (1)(1/0.94 \quad 1)]^{-1}$$
$$F_{12} = 0.61$$

And

$$Q/A = q = (2.04 \times 10^{-8} \mathrm{J/cm^2\,hr\,°K^4})(0.61)[(273)^4 \quad (253)^4]$$
$$= 18.1 \mathrm{J/cm^2\,hr}$$

Note that this value is independent of the spacing between the plates and independent of pressure. We previously estimated the conductive contributions at 1000 mT (viscous) and 1 mT (molecular) as 128 and 1.7 J/cm^2 hr, respectively. The actual heat transfer by thermal conduction will be somewhere in between these values, so we can conclude that the contribution of thermal radiation is less than the conductive component, but should not be ignored. Given that thermal radiation increases with the fourth power of temperature, it will become relatively more important at higher shelf temperatures.

Thermal radiation becomes more significant in light of warm surfaces in proximity to the product, such as the chamber walls and, particularly, the door of the freeze dryer. Thermal radiation has been shown to be an important contributor to the "edge effect" in freeze-drying, where the vials at the edge of an array of vials dry at a significantly higher rate than vials away from the edge (Fig. 13). Rambhatla and co-workers (26) studied this by sputter coating vials with gold to substantially decrease the thermal emissivity of the glass (note that glass has an unusually high thermal emissivity). Sublimation rate was measured gravimetrically for gold-coated versus uncoated vials both at the edge of the array and at the middle of the array. Three different shelf temperatures were used. In each case, sublimation rate was fastest in uncoated vials at the front (close to the Plexiglass door) of the array. Coated vials at the front of the array underwent sublimation at a rate of about half that of uncoated vials. Differences between coated and uncoated vials were much smaller for vials placed somewhere in the middle of the array, which supports the conclusion that thermal radiation is a major contributor to the edge effect. The data also supports the idea that the edge effect is much more pronounced when freeze-drying at low shelf temperature. This makes sense, since the thermal radiation is coming from the environment outside the freeze dryer, and there is a greater driving force for thermal radiation when the shelf temperature is controlled at a low level.

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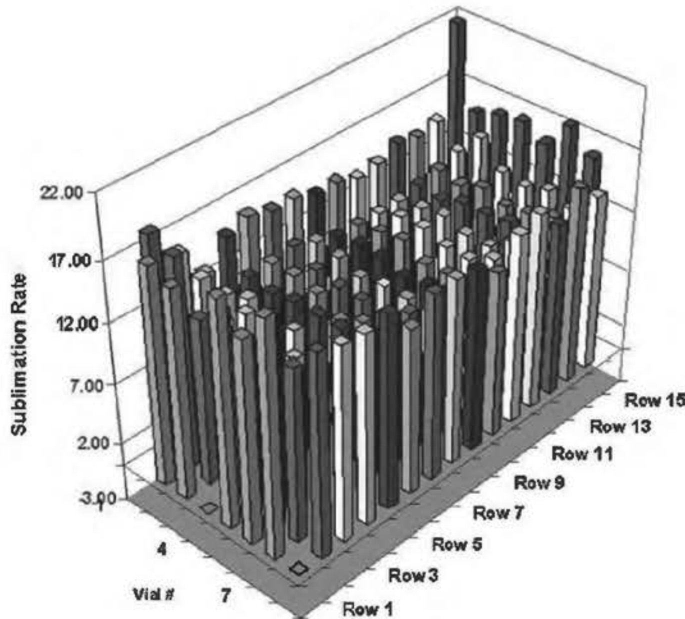

Figure 13 (*See color insert*) Distribution of sublimation rates for a laboratory scale freeze dryer showing the relative mag nitude of "edge effects."

The Vial Heat Transfer Coefficient

The vial heat transfer coefficient is typically measured by filling vials with a representative volume of water and carrying out an abbreviated freeze-drying cycle for sufficient time to sublime perhaps half of the vial contents. A number of vials that were filled, marked for identification, and weighed initially are weighed again after the abbreviated cycle. The heat transfer coefficient is determined using the following equation (27):

$$k_v = q(T_s \quad T_b)$$

where T_b is the temperature at the bottom center of the vial and T_s is the shelf surface temperature. The rate of heat transfer per unit area per unit time, q, is then

$$q = \frac{\Delta H_s \Delta w}{\Delta t}$$

where Δw represents the weight loss of a given vial and Δt is the sublimation time. The vial heat transfer coefficient actually includes three individual terms that reflect underlying heat transfer mechanisms:

$$k_v = k_r + k_c + k_g$$

where k_r is the component due to radiative heat transfer; k_c, the component arising from direct contact between the vial and the shelf; and k_g, the component attributable to conduction through the gas phase resulting from lack of direct contact between vial and shelf. The later term is generally the rate-limiting conductance and is expressed as

$$k_g = \frac{\alpha \Lambda_0 P}{1 + l(\alpha \Lambda_0 / \lambda_0) P}$$

where λ_0 represents the thermal conductivity of water vapor, l represents the average separation distance between the bottom of the vial and the shelf, and the other terms are defined earlier. This expression takes into account heat transfer arising from both flow regimes viscous and molecular flow as a function of the average separation distance. When

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the term $l(\alpha\Lambda_0/\lambda_0)P$ is much larger than unity, then viscous flow conditions apply and the gas conduction term reduces to

$$k_g = \frac{\lambda_0}{l}$$

That is, the conduction term is directly related to the thermal conductivity of water vapor, inversely related to the separation distance, and is independent of pressure. At very small separation distances, where $l(\alpha\Lambda_0/\lambda_0)P \ll 1$, then molecular flow conditions apply and

$$k_g = \alpha\Lambda_0 P$$

That is, the conduction term is independent of separation distance and is directly dependent on pressure.

Measurement of the vial heat transfer coefficient is a useful way to evaluate the effect of changing vial specifications on the suitability of a freeze dry cycle for a given formulation. If the vial heat transfer coefficients are not significantly different, then there should be no effect. A significant difference would require re-examination of the freeze dry cycle.

Mass Transfer in Freeze-Drying

In the same way that there is a series of resistances to heat transfer from the shelf to the sublimation front during primary drying, there is also a series of resistances to mass transfer from the sublimation front to condensation of water vapor on the condenser. These resistances are typically the partially dried product layer, the headspace of the vial including the slot, or slots, in the partially seated stopper, and the resistance associated with the flow of water vapor in the chamber, the duct connecting the chamber with the condenser, and the condenser itself. Not surprisingly, the limiting resistance is almost always the porous bed of partially dried solids. The resistance associated with the vial headspace/stopper is generally quite low, assuming that the stopper is appropriately positioned. The resistance of the chamber/ condenser can, under very aggressive drying conditions, become a controlling resistance because of choked flow, discussed in the following text.

The sublimation rate again takes the form of a flow term equaling a driving force divided by a resistance:

$$\text{Sublimation rate} = \frac{P_i - P_c}{R_p}$$

Where P_i is the vapor pressure of ice at the sublimation front; P_c, the partial pressure of water vapor in the chamber; and R_p, the resistance of the partially dried layer of solids. Since the sublimation front moves from the top of the vial to the bottom during primary drying, the depth of the partially dried layer increases and the resistance increases. This causes the sublimation rate to decrease and, since the rate of heat flow from the shelf remains approximately constant, the product temperature increases. Of course, this also increases P_i and increases the driving force for sublimation. The increased driving force does not completely offset the increased resistance, however, which explains why the product temperature tends to increase gradually during primary drying. Thus, under the same set of primary drying conditions, the product temperature profile can vary widely depending on the resistance characteristics of the formulation. This is illustrated in Figure 14. As resistance to mass transfer increases, control of product temperature becomes more uncertain. For formulations that have a relatively high resistance to flow of water vapor, it is important to limit the depth of the fill. It is generally good practice to limit the fill volume to no more than about one third of the capacity of the vial.

Searles et al. reported that primary drying rate is affected by the nucleation temperature of ice, where high degrees of supercooling result in more rapid freezing once ice crystals nucleate. Fast freezing results in small ice crystals that, in turn, have a relatively high resistance to mass transfer. Conversely, low degrees of supercooling result in larger ice crystals, relatively low resistance to vapor flow, and higher sublimation rate. One benefit of annealing, as reported by Searles et al. (8), is to allow Ostwalt ripening of smaller ice crystals such that a more

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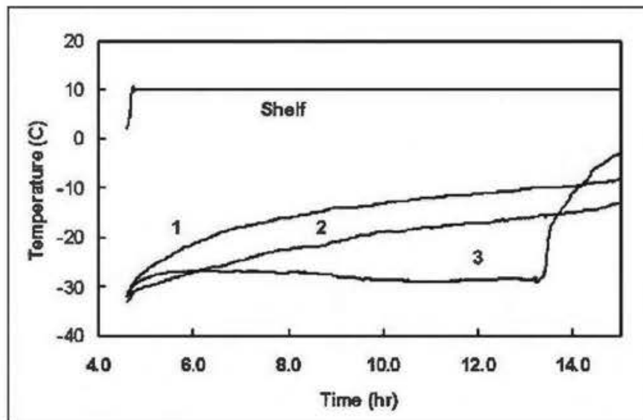

Figure 14 Product temperature versus time during primary drying for (1) 8% mannitol, (2) 4% man nitol, and (3) 5% lactose.

uniform distribution of ice crystal sizes results, with faster average sublimation rate and better vial-to-vial uniformity of sublimation.

There is a need in freeze-drying technology development for better control of the freezing step. This would not only make the freezing step more efficient, but would also improve consistency of drying. Approaches to improved control of freezing include the use of ultrasound (28), an electric field (29), and freezing under a slight vacuum (30). The practical application of any of these techniques has yet to be established however. As of this writing, Praxair, Inc., has reported a technique for controlled nucleation of ice that could be readily scalable, but there appear to be no publications as yet describing the details of the technique.

Change in morphology of the partial dried cake during primary drying can result in a change in resistance. "Microcollapse" of lactose during freeze-drying was reported by Milton and Nail (31), where scanning electron microscopy was used to study the microstructure of the solids. Microcollapse results in holes appearing in plates of amorphous substance, with an accompanying decrease in resistance of the dried layer. This would be expected to result in an increased sublimation rate and a decrease in product temperature during primary drying.

Measurement of Sublimation Rate

The sublimation rate can be measured in several ways. If a sample thief is available to remove samples from the freeze-dryer during the process, then several vials can be pre-weighed and identified. The thief is then used to remove these vials at various times during the primary drying process, reweighed, and a weight loss versus time curve is constructed. If no thief is available, the same approach can be used except that the cycle is terminated before primary drying is completed. Of course, this is a destructive test, and only one drying time point is possible, but the vials can be reweighed and an average sublimation rate over the time interval can be calculated. The most sophisticated method of measuring the sublimation rate is to use tunable diode laser absorption spectroscopy (TDLAS), which is discussed below.

Mass Transfer During Secondary Drying

Secondary drying refers to removal of water that did not freeze during the freezing process. The amount of this unfrozen water depends largely on the nature of the formulation. In formulations with a relatively high content of amorphous solid, the unfrozen water level is relatively high. Since ice is no longer present during secondary drying, higher shelf temperatures are generally used as compared with primary drying. However, for amorphous formulations in particular, collapse can take place during secondary drying if the shelf temperature is increased too rapidly, or to a temperature that is too high.

There is not a large body of published information on secondary drying. Pikal and co-workers (32) studied the rate of secondary drying as a function of shelf temperature and

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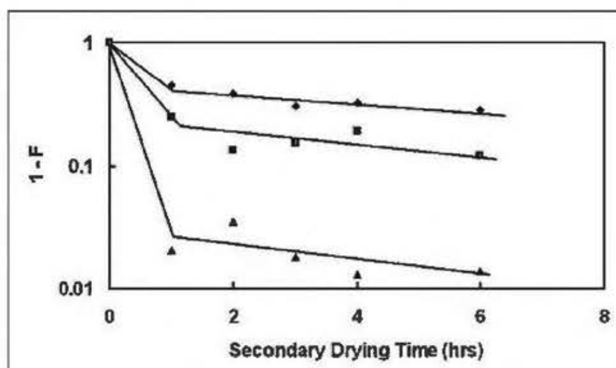

Figure 15 Relative rates of second ary drying for different materials.

chamber pressure for various formulations, and representative results are shown in Figure 15. The rate of water removal during secondary drying is determined by, not surprisingly, the formulation, as well as the shelf temperature. For the formulations examined, secondary drying seems to take place in two stages an early "fast" phase, followed by a "slow" phase, where a plateau is reached in the residual moisture as a function of drying time. This plateau level is determined largely by the shelf temperature during secondary drying. Rate of secondary drying was shown to be, at least for the model systems studied, independent of the chamber pressure. This is counter to the common point of view that the chamber pressure should be reduced to the lowest practical attainable level during secondary drying, and supports the idea that the rate-limiting step in secondary drying is either diffusion of water through the glassy matrix or evaporation of water at the surface of the solid most likely the former.

Choked Flow in Freeze Drying

Normally, the resistance of the dried product layer is the controlling resistance to mass transfer in freeze-drying. However, under aggressive drying conditions, another resistance has been shown to be significant, this one arising from the duct connecting the chamber with the condenser in freeze dryers with external condensers (33). Water vapor flows through this cylindrical duct because the upstream pressure P_u is higher than the downstream pressure, P_d. As water vapor flows through this duct, the pressure decreases and, since the mass flow rate is constant for any axial position along the duct, the velocity of the vapor increases. The kinetic theory of gases shows, however, that there is a limit to the vapor velocity, which is the speed of sound in water vapor, about 360 m/sec or Mach 1. As the speed of sound is approached, further reduction of the pressure on the condenser side of the duct will cause no change in the mass flow rate through the duct. In this case, flow through the duct is said to be *choked*. This represents the maximum sublimation rate that the freeze dryer will support at any given chamber pressure. Attempting to operate at a higher sublimation rate would result in the inability to control chamber pressure.

The choke point is a function of chamber pressure the higher the chamber pressure, the higher the choke point. The choke point can be determined by testing the system using ice slabs, where tray rings are lined with plastic and filled with perhaps 1 to 2 cm of water. All of the shelves are utilized for this. The water is frozen and the system is evacuated. Starting at the low end of the operating pressure range for the freeze dryer, say 50 mT, the pressure is allowed to stabilize, then the shelf temperature is increased, either by ramping the temperature or by making stepwise increases in the shelf temperature set point. A temperature will be reached where the chamber pressure will drift above the set point. This is the choke point for that pressure. A new set pressure set point is then established, and the process is repeated until the operating pressure range has been covered. The actual sublimation rate at each point would need to be determined gravimetrically; that is, by carrying out a brief sublimation cycle and determining the weight of ice sublimed, then converting to an averaged sublimation rate.

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PROCESS MONITORING

The traditional method of monitoring the status of the product is to place a thermocouple, or another temperature measuring device such as a resistance temperature detector (RTD), in several vials of product. This technique provides important information on product temperature during primary drying, when primary drying is complete, as well as an indication of the end point of secondary drying (Fig. 3). While this is necessary when developing freeze dry cycles, it has significant drawbacks as a monitoring method in a manufacturing setting. First, monitored vials are not truly representative of nonmonitored vials, since the temperature measuring device promotes heterogeneous nucleation of ice. This results in lower degrees of supercooling, larger ice crystals, and faster drying rates. In general, monitored vials undergo sublimation at a rate roughly 10% faster than nonmonitored vials. Second, placing thermocouple probes in individual vials is a manual process that inevitably compromises sterility assurance. Some manufacturers try to avoid this by placing monitored vials in the front row of vials, closest to the chamber door. However, as discussed earlier, this position is the most subject to the "edge effect", making data from monitored vials even more nonrepresentative. Finally, advancing technology in parenteral manufacturing has made automated loading/unloading systems common in freeze-drying. Such systems are not compatible with placing temperature measuring devices in individual vials.

There is a continuing need in the industry for better process monitoring, and the past several years have seen considerable activity in process monitoring tools. Below is a brief survey of methods intended to monitor the status of the entire batch.

Comparative Pressure Measurement

Comparative pressure measurement is based on the use of two types of pressure sensors a capacitance manometer and a thermal conductivity-type gauge (a thermocouple gauge or, more commonly, a Pirani gauge) (34). The capacitance manometer is based on capacitance changes associated with a flexible metal diaphragm between a sealed reference cell and the process gases. Thus, it measures force per unit area independently of gas phase composition. The thermocouple-type gauge, on the other hand, is preferentially sensitive to water vapor because of the higher thermal conductivity of water vapor relative to nitrogen or oxygen. In comparative pressure measurement, chamber pressure is both monitored and controlled with the capacitance manometer while it is also monitored with the thermal conductivity-type gauge. A representative graph of a cycle monitored with comparative pressure measurement is shown in Figure 16. During primary drying, the apparent pressure as measured by a Pirani gauge is nearly constant, and is considerably higher than the "true" pressure as measured by capacitance manometer, since the composition of the vapor in the chamber is nearly all water vapor. As primary drying ends and the partial pressure of water vapor decreases, the Pirani reading decreases. As the shelf temperature is increased during secondary drying, the Pirani pressure increases again as unfrozen water is released from the partially dried solids. The

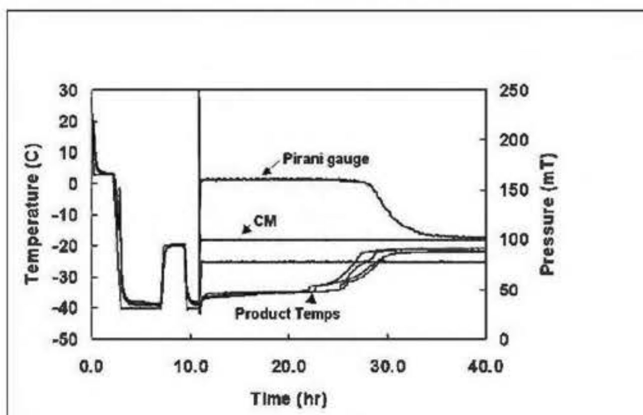

Figure 16 Process variables illustrating monitoring by comparative pressure measurement.

pressure decreases again and approaches a steady-state value near the capacitance manometer reading as the product approaches dryness.

Comparative pressure measurement has proven to be a robust method of monitoring the status of the entire batch. It is independent of scale of operations and is inexpensive. Despite these advantages, it has been rather slow to be adopted by the industry.

Electronic Hygrometer

Electronic hygrometers measure the dew point, or frost point, of a process gas, and are based on either an optical measurement or a capacitance measurement (35). While not commonly used in freeze-drying, the instrument demonstrated to be successful for monitoring freeze-drying is based on capacitance changes due to sorption of water vapor. The process data using the electronic hygrometer is qualitatively very similar to the data shown in Figure 16.

Pressure Rise

The pressure rise technique consists simply of closing the valve between the chamber and condenser for a brief interval as the end of drying is approached. As the rate of water vapor evolution from the product decreases, the amount of pressure rise approaches the background leak rate of the chamber/condenser. The method is simple and robust, but must be applied with some caution. If the sequence of opening the valve periodically begins during primary drying, it is important to assure that the valve does not stay closed so long that the high chamber pressure causes damage to the product.

Manometric Temperature Measurement

Milton and co-workers (31) described a method based on a pressure rise measurement, except that the valve between the chamber and condenser is closed for a brief interval during primary drying. The transient pressure response is measured, and this response is fit to an equation based on fundamental heat and mass transfer consisting of three components: the continued sublimation of ice during the time course of the measurement, continued heat transfer to the vial from the shelf during the measurement, and dissipation of the temperature gradient across the frozen layer during the measurement. The composite equation contains three unknowns: the vapor pressure of ice (thus the temperature at the sublimation front), the resistance of the product to mass transfer, and the vial heat transfer coefficient. A nonlinear least squares algorithm is then used to obtain values of these variables that provide the best fit of the equation to the actual transient pressure response. Using the manometric temperature measurement (MTM) method, reasonable agreement has been observed between product temperatures measured by MTM and those measured by traditional methods like the thermocouple, particularly given that they do not measure the temperature in the same location. Thermocouple measurements typically measure the temperature at the bottom center of the vial, whereas MTM calculates the temperature at the sublimation front, and there is a temperature gradient across the frozen layer of 1 to 2°C. Likewise, reasonable values are obtained for the resistance of the dried product layer to flow of water vapor, and this has led to a better understanding of the role of dried layer morphology on resistance of the solid layer to mass transfer, as discussed earlier. This technique has been advanced further as commercially available software, the SMART® Freeze Dryer, to control the product temperature at the desired value, thus decreasing the amount of trial and error experiments needed in cycle development (36).

While manometric temperature measurement is a very useful laboratory tool, it requires a quick-acting valve between the chamber and condenser to record the transient pressure response appropriately. Since the main valves on production scale freeze dryers have relatively slow-acting valves, the method has not yet been applied to freeze-drying on a commercial scale.

Tunable Diode Laser Absorption Spectroscopy

Tunable diode laser absorption spectroscopy, or TDLAS, is a new and still developing technology that shows significant promise as a process analytical technology in freeze-drying. TDLAS is a near-infrared method that provides real-time measurement of the mass flow rate of water vapor flowing from the chamber to the condenser during freeze-drying. The hardware is

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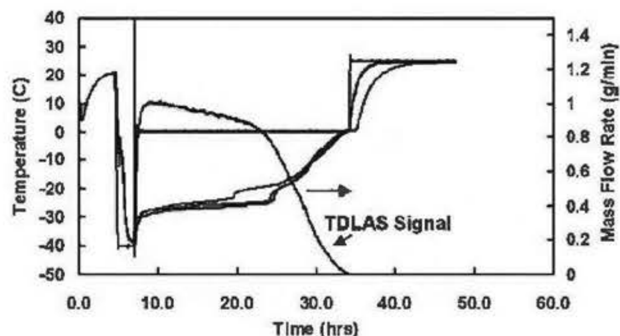

Figure 17 Representative graph of freeze drying process variables showing mass flow rate as measured by tunable diode laser absorption spectroscopy (TDLAS).

mounted on the duct connecting the chamber to the condenser and consists of a fiber-optic laser source aligned at about a 45° angle to a detector on the opposite side of the duct. Water vapor concentration is measured by traditional absorption spectroscopy. The velocity measurement is based on the fact that moving water vapor has a frequency of maximum absorption that is shifted relative to stationary water vapor by an amount that is proportional to the speed of the vapor. The λ_{max} of the moving vapor is compared with that of stationary water vapor sealed in a reference cell. The calculation of average velocity is based on a computational fluid dynamic model of vapor flow in the duct. The velocity measurement, along with the concentration of water vapor, is used to calculate the instantaneous mass flow rate. The instantaneous flow rate data is integrated over the time course of the freeze dry cycle to give the cumulative amount of water removed.

TDLAS has been shown to be a useful tool in cycle development (37). For example, the influence of pressure on sublimation rate can be quantitated simply by changing the set point pressure and observing the resulting sublimation rate. Freeze dryer capability can be readily measured by determining the maximum sublimation rate supported. Measuring capability of both laboratory and production freeze dryers facilitates scale-up by preventing development of aggressive cycles on laboratory equipment that cannot be supported by production scale equipment. Finally, accurate cycle end points can be determined by observing the time at which the flow rate approaches zero (Fig. 17).

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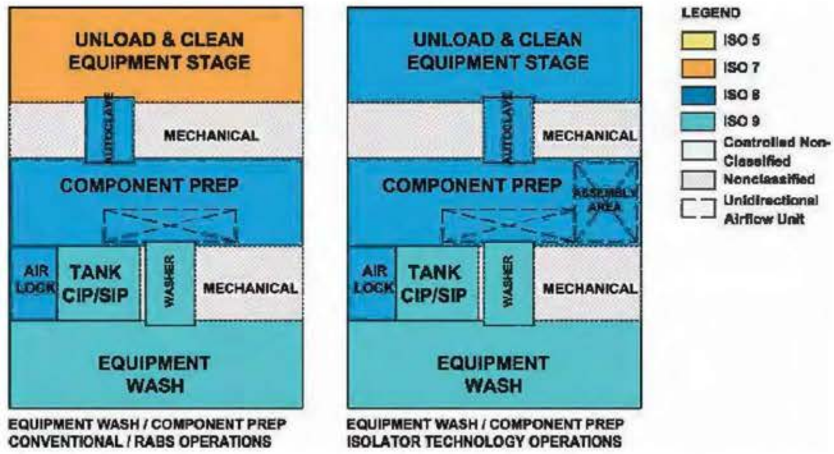

Figure 1.1 Weigh and dispense (*see page 5*).

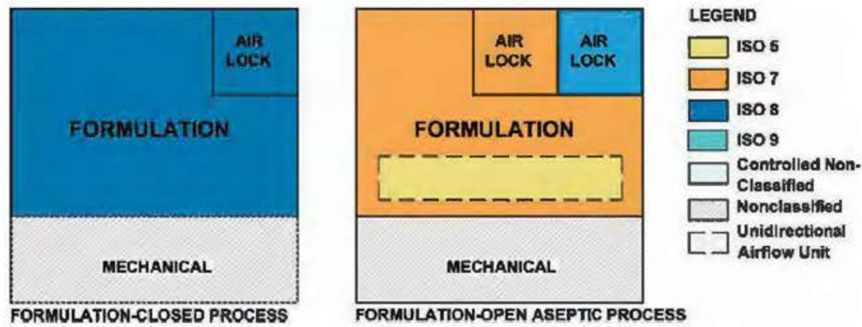

Figure 1.2 Formulation (*see page 7*).

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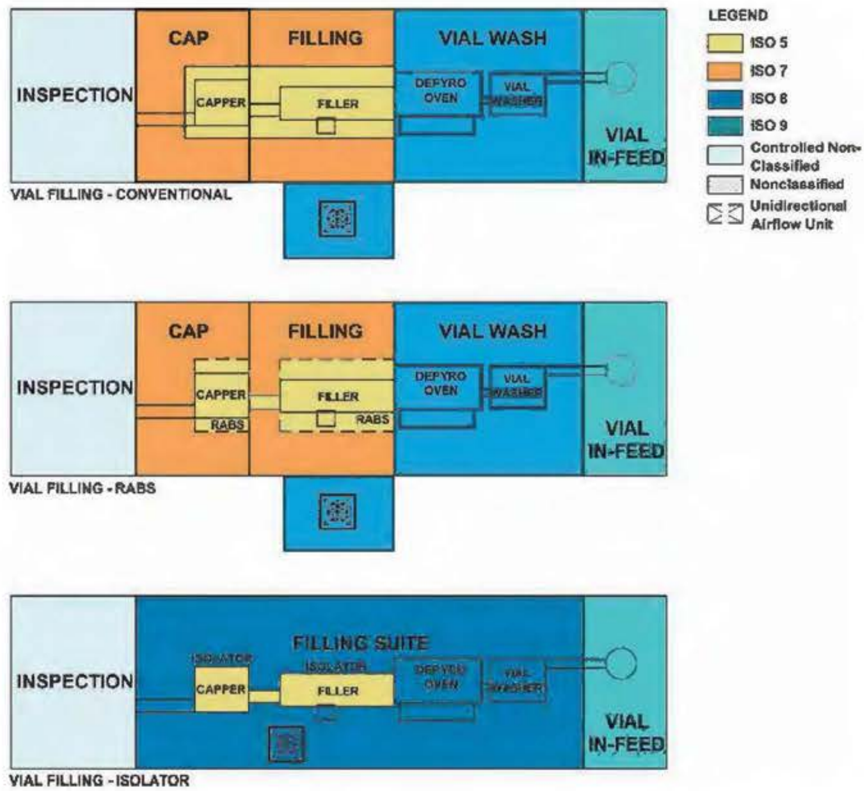

Figure 1.4 Vial filling (*see page 10*).

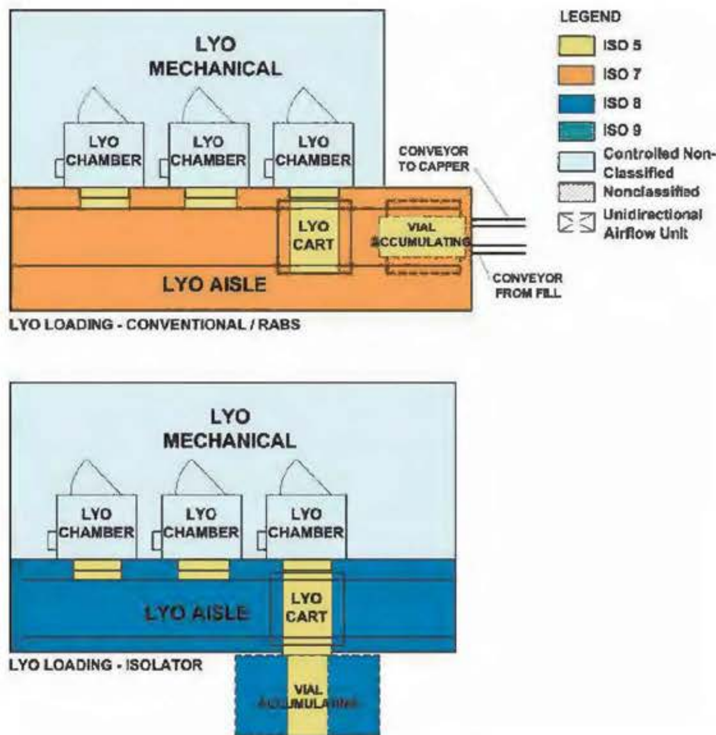

Figure 1.10 LYO load/unload (*see page 18*).

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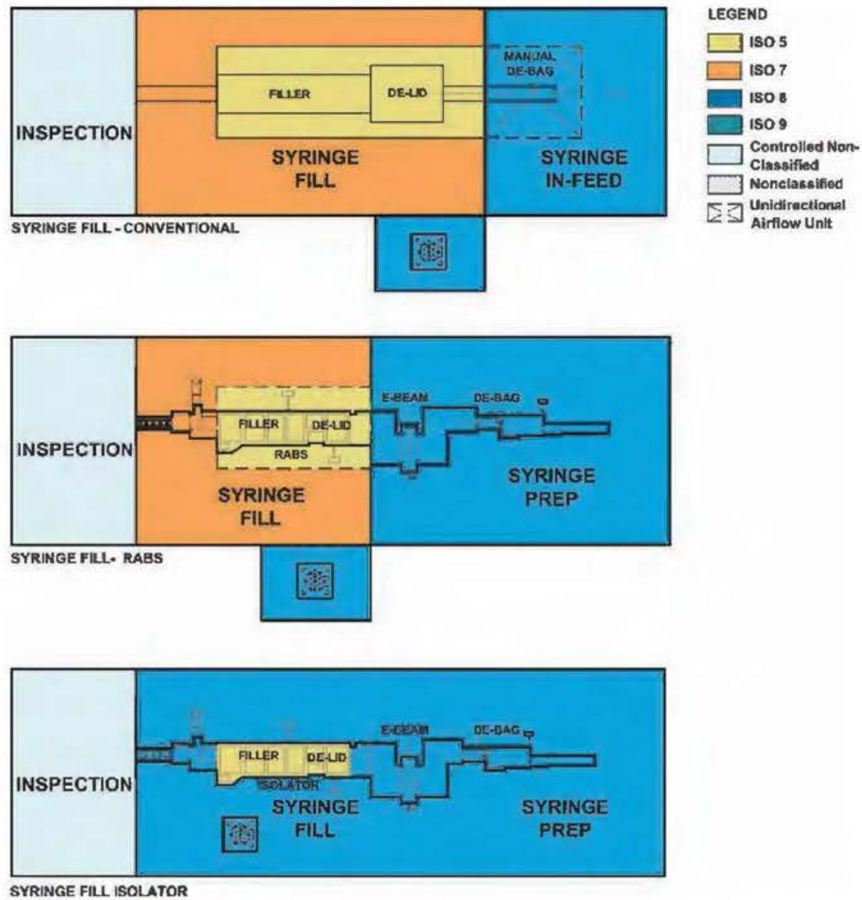

Figure 1.15 Syringe fill (*see page 22*).

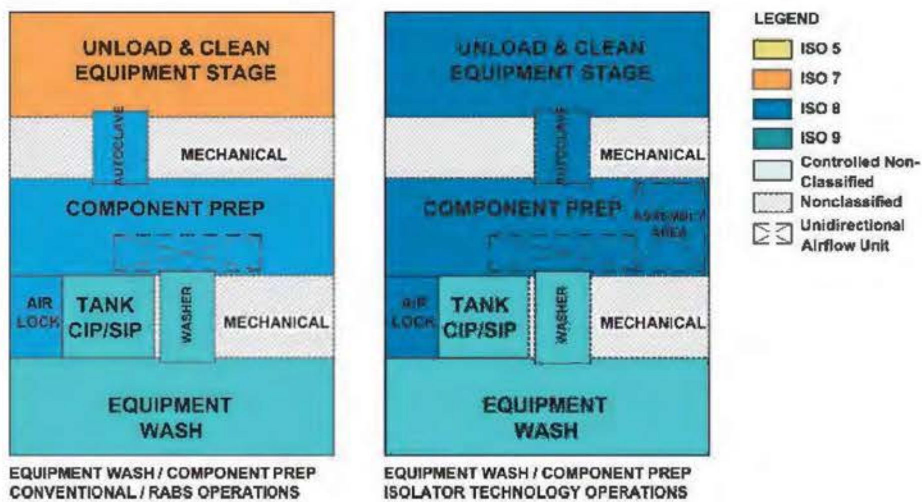

Figure 1.23 Equipment wash/component prep (*see page 30*).

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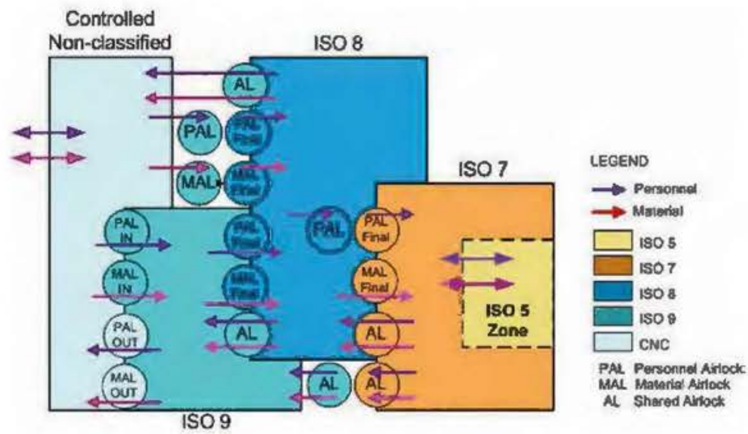

Figure 1.26 General airlock concept by classification (*see page 40*).

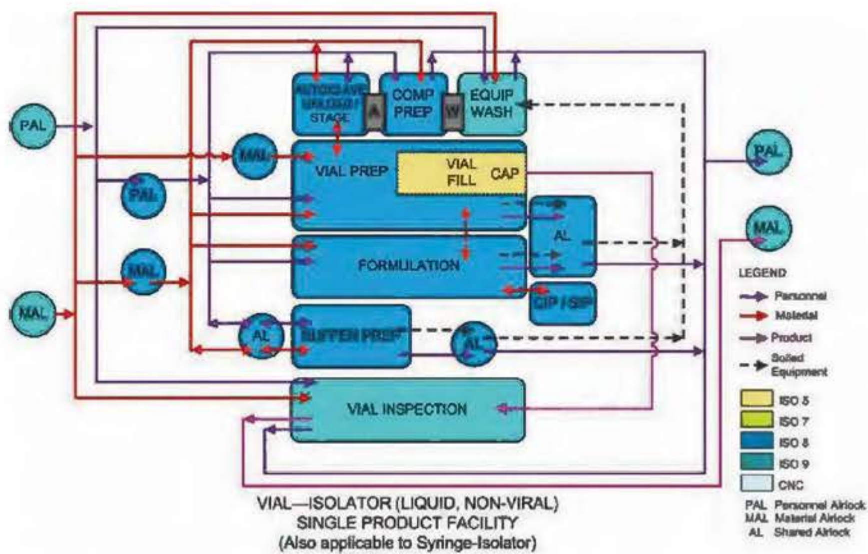

Figure 1.27 Single product single suite module (*see page 41*).

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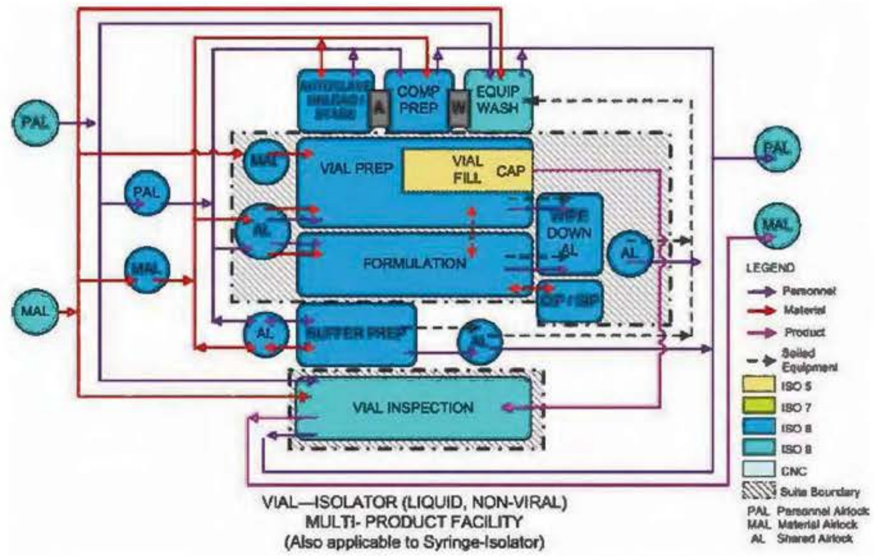

Figure 1.28 Multiproduct multi suite module (*see page 42*).

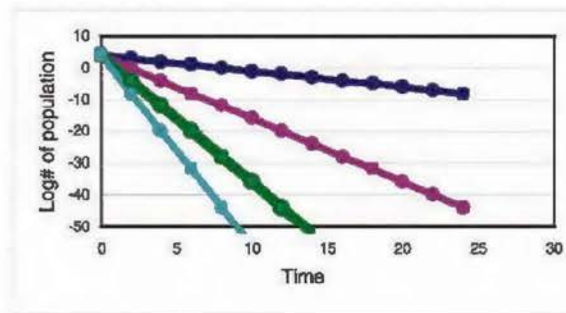

Figure 9.15 Overkill approach (*see page 231*).

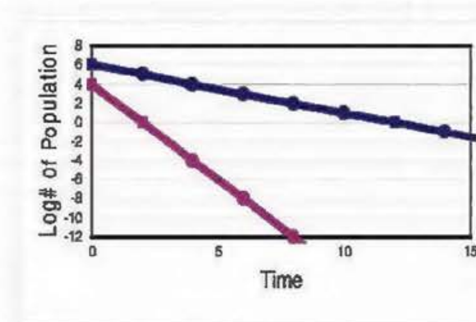

Figure 9.16 BB/BI method with survival count (*see page 232*).

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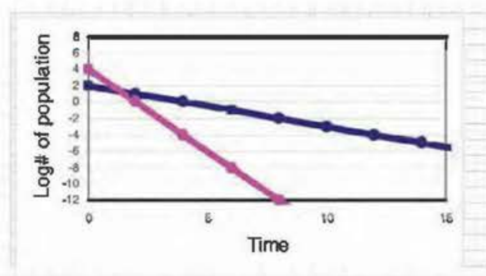

Figure 9.17 BB/BI method with total BI kill (*see page 232*).

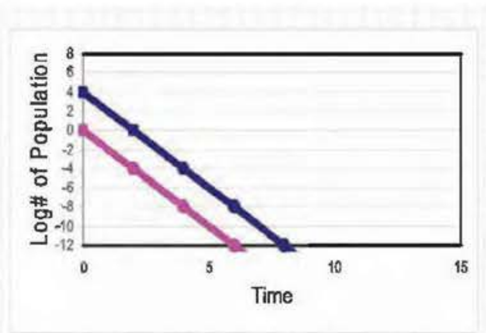

Figure 9.18 Bioburden approach (*see page 233*).

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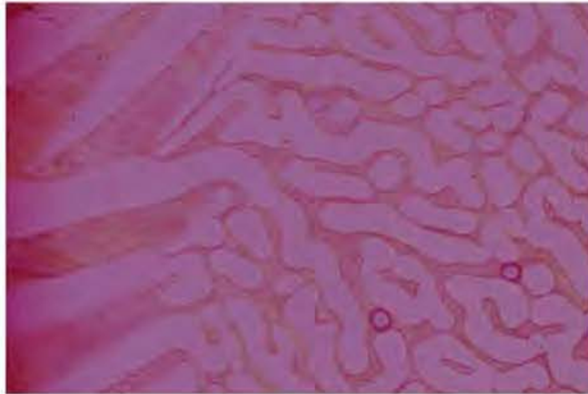
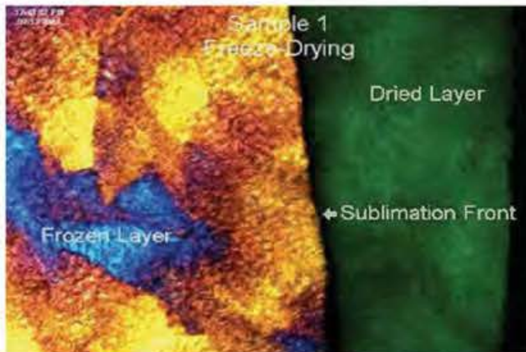


Figure 15.6 Photomicrograph of a fro zen solution of sodium chloride in water (*see page 361*).



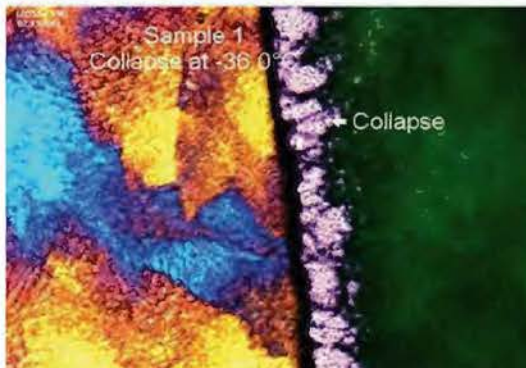


Figure 15.11 Photomicrographs taken during freeze drying showing retention of structure (*top*) and collapse (adjacent to sublimation front) (*see page 367*).

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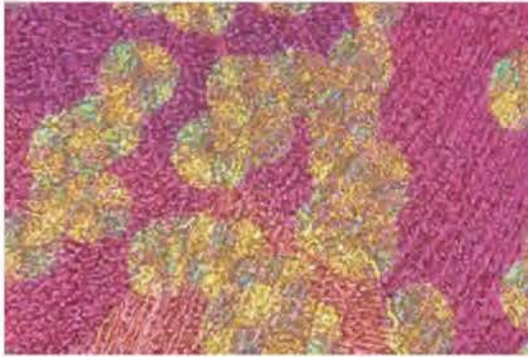

Figure 15.12 Photomicrograph showing crys tallization of a solute from a frozen system during annealing (*see page 368*).

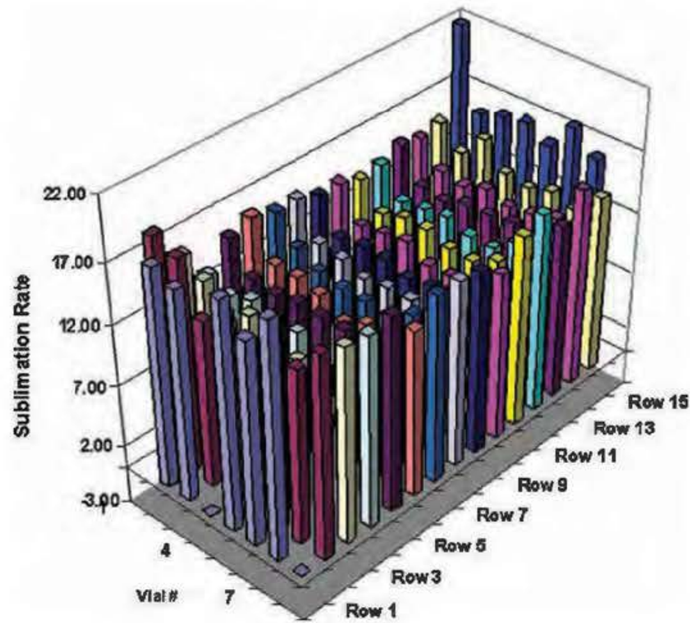

Figure 15.13 Distribution of sublimation rates for a laboratory scale freeze dryer showing the relative magnitude of "edge effects" (*see page 374*).

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Pharmaceutical Dosage Forms: Parenteral Medications
Third Edition
Volume 2: Facility Design, Sterilization and Processing

About the book

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

First published in 1984 (as two volumes) and then last revised in 1993 (when it grew to three volumes), this latest revision will address the plethora of changes in the science and considerable advances in the technology associated with these products and routes of administration. The third edition of this book maintains the features that made the last edition so popular but comprises several brand new chapters, revisions to all other chapters, as well as high quality illustrations.

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- A comprehensive chapter on pharmaceutical water systems.
- A discussion of quality attributes of sterile dosage forms, including particulate matter, endotoxin, and sterility testing.
- A detailed chapter on processing of parenteral drug products (SVPs and LVPs).
- Presentations on widely used sterilization technologies – steam, gas / chemical, radiation, filtration and dry heat.
- An in-depth chapter on lyophilization.

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