A Guide To Freeze Drying for the Laboratory

Protecting your laboratory environment

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Foreword

This booklet has been developed to serve as a basic guide to the freeze drying process. The information presented is generic in nature and is the result of research and experience by Labconco personnel and users of freeze drying equipment. It is our intention to provide a non-biased review of preparation techniques and freeze drying methods. The purpose of this booklet is to help you make an informed choice of equipment for your laboratory applications.

Our Method

We begin our discussion of freeze drying for the laboratory by examining the three steps in the process: prefreezing, primary drying and secondary drying. Next, we examine a typical freeze drying cycle and the methods available to facilitate the freeze drying process using equipment designed for use by laboratories. Finally, suggestions to optimize successful results are discussed, including determination of end point, contamination, backfilling of dried samples and product stability. A glossary of terms used throughout this booklet to explain the freeze drying process follows the text, along with a bibliography.

Introduction

Freeze drying has been used in a number of applications for many years, most commonly in the food and pharmaceutical industries. There are, however, many other uses for the process including the stabilization of living materials such as microbial cultures, preservation of whole animal specimens for museum display, restoration of books and other items damaged by water, and the concentration and recovery of reaction products.

Specialized equipment is required to create the conditions conducive to the freeze drying process. This equipment is currently available and can accommodate freeze drying of materials from laboratory scale projects to industrial production.

Freeze drying involves the removal of water or other solvent from a frozen product by a process called *sublimation.* Sublimation occurs when a frozen liquid goes directly to the gaseous state without passing through the liquid phase. In contrast, drying at ambient temperatures from the liquid phase usually results in changes in the product, and may be suitable only for some materials. However, in freeze drying, the material does not go through the liquid phase, and it allows the preparation of a stable product that is easy to use and aesthetic in appearance.

The advantages of freeze drying are obvious. Properly freeze dried products do not need refrigeration, and can be stored at ambient temperatures. Because the cost of the specialized equipment required for freeze drying can be substantial, the process may appear to be an expensive undertaking. However, savings realized by stabilizing an otherwise unstable product at ambient temperatures, thus eliminating the need for refrigeration, more than compensate for the investment in freeze drying equipment.

Principles of Freeze Drying

The freeze drying process consists of three stages: prefreezing, primary drying, and secondary drying.

Prefreezing: Since freeze drying is a change in state from the solid phase to the gaseous phase, material to be freeze dried must first be adequately prefrozen. The method of prefreezing and the final temperature of the frozen product can affect the ability to successfully freeze dry the material.

Rapid cooling results in small ice crystals, useful in preserving structures to be examined microscopically, but resulting in a product that is more difficult to freeze dry. Slower cooling results in larger ice crystals and less restrictive channels in the matrix during the drying process.

Products freeze in two ways, depending on the makeup of the product. The majority of products that are subjected to freeze drying consist primarily of water, the *solvent,* and the materials dissolved or suspended in the water, the *solute.* Most samples that are to be freeze dried are *eutectics* which are a mixture of substances that freeze at lower temperatures than the surrounding water. When the aqueous suspension is cooled, changes occur in the solute concentrations of the product matrix. And as cooling proceeds, the water is separated from the solutes as it changes to ice, creating more concentrated areas of solute. These pockets of concentrated materials have a lower freezing temperature than the water. Although a product may appear to be frozen because of all the ice present, in actuality it is not completely frozen until all of the solute in the suspension is frozen. The mixture of various concentration of solutes with the solvent constitutes the eutectic of the suspension. Only when all of the eutectic mixture is frozen is the suspension properly frozen. This is called the *eutectic temperature.*

It is very important in freeze drying to prefreeze the product to below the eutectic temperature before beginning the freeze drying process. Small pockets of unfrozen material remaining in the product expand and compromise the structural stability of the freeze dried product.

The second type of frozen product is a suspension that undergoes *glass formation* during the freezing process. Instead of forming eutectics, the entire suspension becomes increasingly viscous as the temperature is lowered. Finally the product freezes at the *glass transition point* forming a vitreous solid. This type of product is extremely difficult to freeze dry.

Primary drying: Several factors can affect the ability to freeze dry a frozen suspension. While these factors can be discussed independently, it must be remembered that they interact in a dynamic system, and it is this delicate balance between these factors that results in a properly freeze dried product.

After prefreezing the product, conditions must be established in which ice can be removed from the frozen product via sublimation, resulting in a dry, structurally intact product. This requires very careful control of the two parameters, temperature and pressure, involved in the freeze drying system. The rate of sublimation of ice from a frozen product depends upon the difference in

vapor pressure of the product compared to the vapor pressure of the ice collector. Molecules migrate from the higher pressure sample to a lower pressure area. Since vapor pressure is related to temperature, it is necessary that the product temperature is warmer than the cold trap (ice collector) temperature. It is extremely important that the temperature at which a product is freeze dried is balanced between the temperature that maintains the frozen integrity of the product and the temperature that maximizes the vapor pressure of the product. This balance is key to optimum drying. The typical phase diagram shown in Figure 1 illustrates this point. Most products are frozen well below their eutectic or glass transition point (Point A), and then the temperature is raised to just below this critical temperature (Point B) and they are subjected to a reduced pressure. At this point the freeze drying process is started.

Figure 1
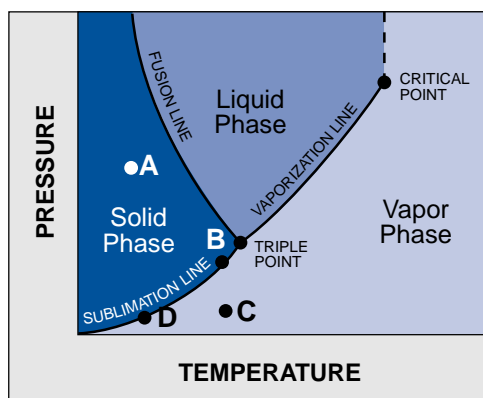
A typical phase diagram.

Some products such as aqueous sucrose solutions can undergo structural changes during the drying process resulting in a phenomenon known as *collapse.* Although the product is frozen below its eutectic temperature, warming during the freeze drying process can affect the structure of the frozen matrix at the boundary of the drying front. This results in a collapse of the structural matrix. To prevent collapse of products containing

A vacuum pump is essential to evacuate the environment around the product to be freeze dried.

sucrose, the product temperature must remain below a critical *collapse temperature* during primary drying. The collapse temperature for sucrose is -32° C.

No matter what type of freeze drying system is used, conditions must be created to encourage the free flow of water molecules from the product. Therefore, a vacuum pump is an essential component of a freeze drying system, and is used to lower the pressure of the environment around the product (to Point C). The other essential component is a collecting system, which is a cold trap used to collect the moisture that leaves the frozen product. The collector condenses out all condensable gases, i.e; the water molecules, and the vacuum pump removes all non-condensable gases.
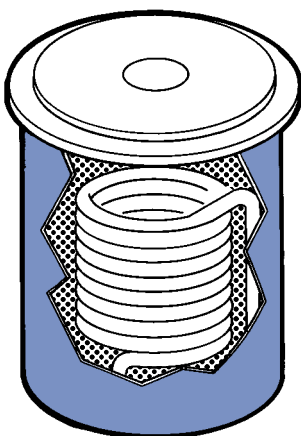
The collecting system acts as a cold trap to collect moisture leaving the frozen product.

It is important to understand that the vapor pressure of the product forces the sublimation of the water vapor molecules from the frozen product matrix to the collector. The molecules have a natural affinity to move toward the collector because its vapor pressure is lower than that of the product. Therefore, the collector temperature (Point D) must be significantly lower than the product temperature. As can be noted in Table 1, raising the product temperature has more effect on the vapor pressure differential than lowering the collector temperature.

Table 1

Vapor Pressure (mBar)	Temperature (°C)
6.104	0
2.599	-10
1.034	-20
0.381	-30
0.129	-40
0.036	-50
0.011	-60
0.0025	-70
0.0005	-80

Vapor Pressure/Temperature Relationships

A third component essential in a freeze drying system is energy. Energy is supplied in the form of heat. Almost ten times as much energy is required to sublime a gram of water from the frozen to the gaseous state as is required to freeze a gram of water. Therefore, with all other conditions being adequate, heat must be applied to the product to encourage the removal of water in the form of vapor from the frozen product. The heat must be very carefully controlled, as applying more heat than the evaporative cooling in the system can remove warms the product above its eutectic or collapse temperature.

Heat can be applied by several means. One method is to apply heat directly through a thermal conductor shelf such as is used in tray drying. Another method is to use ambient heat as in manifold drying.

Secondary drying: After primary freeze drying is complete, and all ice has sublimed, bound moisture is still present in the product. The product appears dry, but the residual moisture content may be as high as 7-8%. Continued drying is necessary at the warmer temperature to reduce the residual moisture content to optimum values. This process is called *isothermal desorption* as the bound water is desorbed from the product.

Secondary drying is normally continued at a product temperature higher than ambient but compatible with the sensitivity of the product. All other conditions, such as pressure and collector temperature, remain the same. Because the process is desorptive, the vacuum should be as low as possible (no elevated pressure) and the collector temperature as cold as can be attained. Secondary drying is usually carried out for approximately 1/3 to 1/2 the time required for primary drying.

How Freeze Drying Works

Refer to the phase diagram (Figure 1) and a typical sublimation cycle (Figure 2). The product is first cooled to below its eutectic temperature (Point A). The collector is cooled to a temperature approximately 20° C cooler than the product temperature, generally around -50 to -80° C. The product should be freeze dried at a temperature slightly lower than its eutectic or collapse temperature (Point B) since the colder the product, the longer the time required to complete primary drying, and the colder the collector temperature required to adequately freeze dry the product.

After the product is adequately frozen and the collector temperature achieved, the system is evacuated using a vacuum pump (Point C). At this point, primary drying of the product begins and continues until the entire frozen matrix appears dry. Heat input to the product may be achieved by several means such as increasing the shelf temperature in the case of tray drying, or using a liquid bath for manifold drying. While the collector and vacuum pump create the conditions for allowing sublimation to occur, heat input is really the driving force behind the whole process.

Heat input to the sample can be enhanced by controlling the pressure in the system at some level above the ultimate capability of the vacuum pump. Some

freeze dryers incorporate vacuum control systems that automatically regulate the pressure to the preset level. This allows additional gas molecules to reside in the system thereby improving the conduction of heat to the sample. This improves the sublimation rate, reducing process time and associated energy costs. Care must be taken to prevent the pressure within the system from exceeding the ice vapor pressure of the product or melting of the sample may occur.

Figure 2
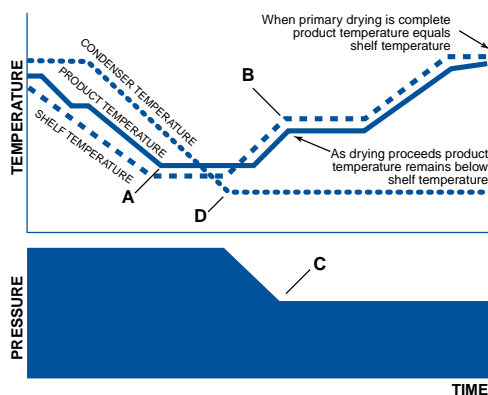
Typical Sublimation Cycle found in system utilizing Tray Dryer with shelves.

Heat input to the product must be very carefully controlled especially during the early stages of drying. The configuration of the product container and the volume of the contained product can affect the amount of heat that can be applied. For small volumes of material, *evaporative cooling* compensates for high levels of heat and drying is accelerated.

The volume and configuration of the suspension to be freeze dried often determines how the material is freeze dried. For example, the greater the ratio of the surface area to the volume of the suspension, the faster drying occurs. This is because a greater area for the water molecules to leave the product exists compared to the distance they have to travel to reach the surface of the frozen matrix. Drying occurs from the top of the product and initially the removal of water molecules is efficient. However, as the drying front moves down through the

Figure 3
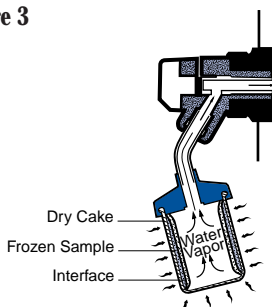
Ambient room temperature provides heat to encourage removal of water vapor from frozen samples when manifold drying.

product, drying becomes more and more difficult. The water molecules must now travel through the dried portions of the product which impedes their progress. As the drying front moves farther and farther down the matrix, the application of heat to the product becomes more important (Figure 3).

Shell freezing as a method of prefreezing the product can increase the surface area to volume ratio by spreading out the frozen product inside the vessel (Figure 4). Shell freezing is accomplished by rotating the vessel in a low temperature bath causing the product to freeze in a thin layer on the inside surface of the vessel. The thickness of the frozen suspension depends on the volume of the product in comparison to the size of the vessel. Shell freezing is primarily used in conjunction with manifold drying.

The vacuum system is very important during freeze drying because the pressure must be maintained at a low level to ensure adequate water vapor flow from the product to the collector. A pressure gauge (commonly called a vacuum gauge) is used to monitor the pressure in the system during the drying process. Pressure can be expressed in several different units which are compared in Table 2. Some gauges measure condensable gases, while others do not. Those gauges that do not measure the condensable gases give an indication of the total pressure in the system. Gauges that do sense the condensable gases indicate a change in pressure during drying. These sensors can be used as an indication of the rate of drying, as well as the endpoint of the drying process.

Figure 4
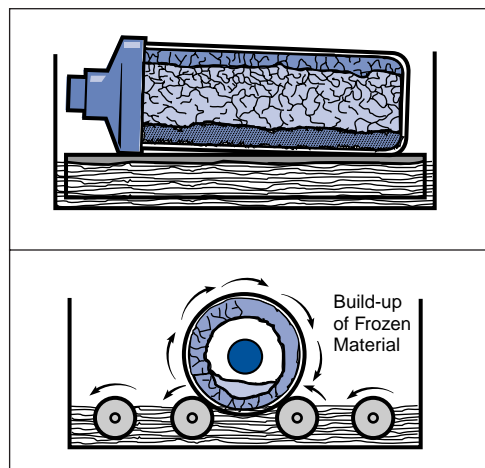
Shell freezing can increase the surface area to volume ratio by spreading out the frozen product inside the vessel.

Table 2

Microns	mm Hg	Torr	mBar
1000	1	1	1.33
100	0.1	0.1	0.133
10	0.01	0.01	0.03

Pressure Relationships

Freeze Drying Methods

Three methods of freeze drying are commonly used: (1) manifold drying, (2) batch drying, and (3) bulk drying. Each method has a specific purpose, and the method used depends on the product and the final configuration desired.

Manifold Method. In the manifold method, flasks, ampules or vials are individually attached to the ports of a manifold or drying chamber. The product is either frozen in a freezer, by direct submersion in a low temperature bath, or by shell freezing, depending on the nature of the product and the volume to be freeze dried. The prefrozen product is quickly attached to the drying chamber or manifold to prevent warming. The vacuum must be created in the product container quickly, and the operator relies on evaporative cooling to maintain the low temperature of the product. This procedure can only be used for relatively small volumes and products with high eutectic and collapse temperatures.

Manifold drying has several advantages over batch tray drying. Since the vessels are attached to the manifold individually, each vial or flask has a direct path to the collector. This removes some of the competition for molecular space created in a batch system, and is most ideally realized in a cylindrical drying chamber where the distance from the collector to each product vessel is the same. In a "tee" manifold, the water molecules leaving the product in vessels farthest from the collector experience some traffic congestion as they travel past the ports of other vessels.

Heat input can be affected by simply exposing the vessels to ambient temperature or via a circulating bath. For some products, where precise temperature control is required, manifold drying may not be suitable.

Several vessels can be accommodated on a manifold system allowing drying of different products at the same time, in different sized vessels, with a variety of closure systems. Since the products and their volumes may differ, each vessel can be removed from the manifold separately as its drying is completed. The close proximity to the collector also creates an environment that maximizes drying efficiency.

Batch Method. In batch drying, large numbers of similar sized vessels containing like products are placed together in a tray dryer. The product is usually prefrozen on the shelf of the tray dryer. Precise control of the product temperature and the amount of heat applied to the product during drying can be maintained. Generally all vials in the batch are treated alike during the drying process, although some variation in the system can occur. Slight differences in heat input from the shelf can be experienced in different areas. Vials located in the front portion of the shelf may be radiantly heated through the clear door. These slight variations can result in small differences in residual moisture.

Batch drying allows closure of all vials in a lot at the same time, under the same atmospheric conditions. The vials can be stoppered in a vacuum, or after backfilling with inert gas. Stoppering of all vials at the same time ensures a uniform environment in each vial and uniform product stability during storage. Batch drying is used to prepare large numbers of ampules or vials of one product and is commonly used in the pharmaceutical industry.
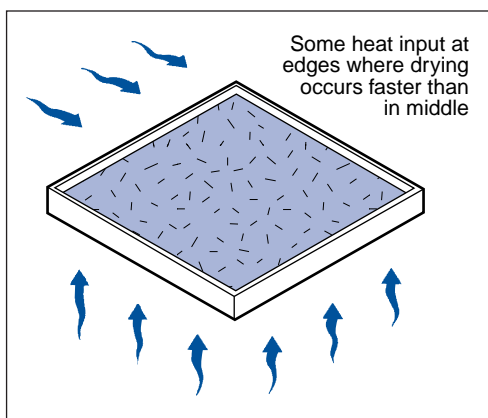

Batch drying in a tray dryer permits precise control of product temperature and heat input.

Bulk Method. Bulk drying is generally carried out in a tray dryer like batch drying. However, the product is poured into a bulk pan and dried as a single unit. Although the product is spread throughout the entire surface area of the shelf and may be the same thickness as product dried in vials, the lack of empty spaces within the product mass changes the rate of heat input. The heat input is limited primarily to that provided by contact with the shelf as shown in Figure 5.

Bulk drying does not lend itself to sealing of product under controlled conditions as does manifold or batch drying. Usually the product is removed from the freeze dry system prior to closure, and then packaged in air tight containers. Bulk drying is generally reserved for stable products that are not highly sensitive to oxygen or moisture.

In the manifold drying method, flasks are individually attached to the ports of a drying chamber.

Figure 5

Some heat input at edges where drying occurs faster than in middle

In bulk drying, heat is provided primarily through conduction from shelf.

Determining Drying Endpoints

Several means can be used to determine the endpoint of primary drying. The drying boundary in batch drying containers has moved to the bottom of the product container and inspection reveals that no ice is visible in the product. No visible ice indicates only that drying at the edges of the container is complete and gives no indication of the conditions in the center of the product. An electronic vacuum gauge can be used to measure condensable gases in the system. When the pressure indicated by the electronic gauge reaches the minimum pressure attainable by the system, as measured by using a McLeod vacuum gauge or as determined previously, no more water vapor is leaving the product.

As the heat input to the product is increased, evaporative cooling keeps the product temperature well below the temperature of its surrounding atmosphere. When primary drying is complete, the product temperature rises to equal the temperature of its environment. In manifold systems and tray dryers with external collectors, the path to the collector can be shut off with a valve and the pressure above the product measured with a vacuum gauge. If drying is still occurring, the pressure in the system increases.

Contamination in a Freeze Dry System

Two types of contamination can occur in a freeze dry system. One results from freeze drying microorganisms and the other results from freeze drying corrosive materials.

The potential for contamination of a freeze drying system by microorganisms is real in any system where microorganisms are freeze dried without a protective barrier such as a bacteriological filter. Contamination is most evident in batch tray dryer systems where large numbers of vials are dried in a single chamber. Evidence for contamination can be found by sampling the surfaces of the vials, shelves and collector. The greatest degree of contamination is usually found on the vials and on the collector. Some vial contamination can be due to a bit of sloppiness in dispensing the material originally, but contamination on the collector is due to microorganisms traveling from the product to the collector through the vapor stream.

The potential for contamination must be considered every time microorganisms are freeze dried, and precautions must be taken in handling material after the freeze dry process is completed. Recognizing that the vials are potentially contaminated, the operator should remove the vials to a safe area such as a laminar flow hood for decontamination. Decontamination of the freeze dry system depends upon the type of freeze dry system used. Some tray dryer systems are designed for decontamination under pressure using ethylene oxide sterilization. Ethylene oxide is considered hazardous, corrosive and detrimental to rubber components. Its use should be carefully monitored.

Coupled with the risk of contamination in a freeze dry system is the risk of cross contamination when freeze drying more than one product at time. It is not a good practice to mix microbiological products in a freeze dry system unless some type of bacteriological filter is used to prevent the microbial product from leaving the vial itself.

While freeze drying of corrosive materials does not necessarily present a risk to the operator, it does present a risk of damaging the freeze dry system itself. Freeze dry systems are designed using materials that resist corrosion and prevent the build up of corrosive materials. But care should be taken to clean the system thoroughly following each use to protect it from damage.

Backfilling

For many freeze dried products, the most ideal system of closure is while under vacuum. This provides an environment in which moisture and oxygen, both detrimental to the freeze dried material, are prevented from coming in contact with the product. In some cases, vacuum in a container may be less than ideal, especially when a syringe is used to recover the product, or when opening the vessel results in a rush of potentially contaminating air. In these cases, backfilling the product container with an inert gas such as argon or nitrogen is often beneficial. The inert gas must be ultrapure, containing no oxygen or moisture.

Backfilling of the product container is generally useful in a batch tray dryer type system. The backfilling should also be carried out through a bacteriological filter. It is important that the gas flow during backfilling be slow enough to allow cooling of the gas to prevent raising the collector temperature. Backfilling can be carried out to any desired pressure in those tray dryers that have internal stoppering capability, and the vials then stoppered at the desired pressure.

Stability of Freeze Dried Products

Several factors can affect the stability of freeze dried material. Two of the most important are moisture and oxygen.

All freeze dried products have a small amount of moisture remaining in them termed *residual moisture.* The amount of moisture remaining in the material depends on the nature of the product and the length of secondary drying. Residual moisture can be measured by several means: chemically, chromatographically, manometrically or gravimetrically. It is expressed as a weight percentage of the total weight of the dried product. Residual moisture values range from <1% to 3% for most products.

By their nature, freeze dried materials are hygroscopic and exposure to moisture during storage can destabilize the product. Packaging used for freeze dried materials must be impermeable to atmospheric moisture. Storing products in low humidity environments can reduce the risk of degradation by exposure to moisture. Oxygen is also detrimental to the stability of most freeze dried material so the packaging used must also be impermeable to air.

The detrimental effects of oxygen and moisture are temperature dependent. The higher the storage temperature, the faster a product degrades. Most freeze dried products can be maintained at refrigerator temperatures, i.e. 4-8° C. Placing freeze dried products at lower temperatures extends their shelf life. The shelf life of a freeze dried product can be predicted by measuring the rate of degradation of the product at an elevated temperature. This is called *accelerated storage.* By choosing the proper time and temperature relationships at elevated temperatures, the rate of product degradation can be predicted at lower storage temperatures.
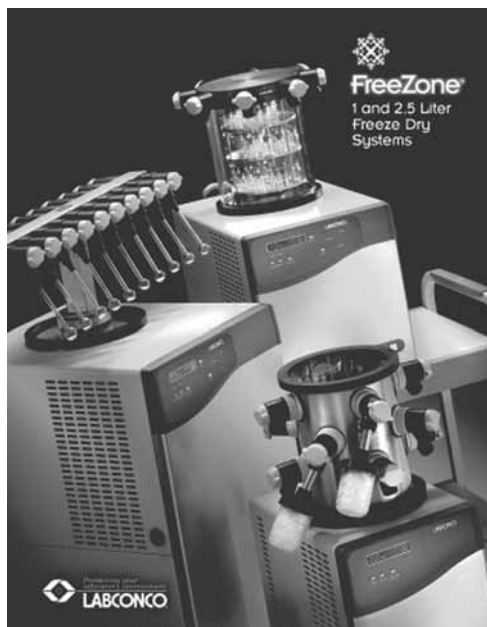
Contact Labconco for a complimentary catalog of Freeze Dry Systems designed for laboratory use.

The Labconco catalog provides additional information about freeze drying equipment. It is available upon request from your laboratory supply dealer or Labconco Corporation at (800) 821-5525, (816) 333-8811 or www.labconco.com.

Glossary

Accelerated Storage: Exposure of freeze dried products to elevated temperatures to accelerate the degradation process that occurs during storage.

Batch Freeze Drying: Freeze drying multiple samples of the same product in similar sized vessels at the same time in a shelf tray dryer.

Bulk Freeze Drying: Freeze drying a large sample of a single product in one vessel such as the bulk drying pans designed for shelf tray dryers.

Collapse: A phenomenon causing collapse of the structural integrity of a freeze dried product due to too high a temperature at the drying front.

Collapse Temperature: The temperature above which collapse occurs.

Collector: A cold trap designed to condense the water vapor flowing from a product undergoing freeze drying.

Internal Collector: A collector located in the same area as the product. All water vapor has a free path to the collector.

External Collector: A collector located outside the product area connected by a small port through which all water vapor must pass. Allows isolation of the product from the collector for drying end point determinations and easier defrosting.

Ethylene Oxide: A colorless, odorless gas used for gas sterilization of tray dryer systems.

Eutectics: Areas of solute concentration that freeze at a lower temperature than the surrounding water. Eutectics can occur at several different temperatures depending on the complexity of the product.

Eutectic Temperature: The temperature at which all areas of concentrated solute are frozen.

Evaporative Cooling: Cooling of a liquid at reduced pressures caused by loss of the latent heat of evaporation.

Freeze Drying: The process of drying a frozen product by creating conditions for sublimation of ice directly to water vapor.

Glass Transition Temperature: The temperature at which certain products go from a liquid to a vitreous solid without ice crystal formation.

Isothermal Desorption: The process of desorbing water from a freeze dried product by applying heat under vacuum.

Lyophilization: The freeze drying process.

Manifold Freeze Drying: A freeze drying process where each vessel is individually attached to a manifold port resulting in a direct path to the collector for each vessel.

Prefreezing: The process of cooling a product to below its eutectic temperature prior to freeze drying.

Pressure Gauge (Vacuum Gauge): An instrument used to measure very low pressures in a freeze drying system.

Thermocouple Gauge: A pressure gauge that measures only the condensable gases in the system. This gauge can be used as an indicator of drying end points.

McLeod Gauge: A mercury gauge used to measure total pressure in the system (i.e. condensable and non-condensable gases.)

Primary Drying: The process of removing all unbound water that has formed ice crystals in a product undergoing freeze drying.

Residual Moisture: The small amount of bound water that remains in a freeze dried product after primary drying. Residual moisture is expressed as the weight percentage of water remaining compared to the total weight of the dried product. The amount of residual moisture in a freeze dried product can be reduced during secondary drying.

Secondary Drying: The process of reducing the amount of bound water in a freeze dried product after primary drying is complete. During secondary drying, heat is applied to the product under very low pressures.

Shell Freezing: Freezing a product in a thin layer that coats the inside of the product container. Shell freezing is accomplished by swirling or rotating the product container in a low temperature bath.

Sublimation: The conversion of water from the solid state (ice) directly to the gaseous state (water vapor) without going through the liquid state.

Vapor Pressure: The pressure of the vapor in equilibrium with the sample.

Bibliography

1. Barbaree, J.M. and A. Sanchez. 1982. Cross-contamination during lyophilization. *Cryobiology* 19:443-447.

2. Barbaree, J.M., A. Sanchez and G.N. Sanden. 1985. Problems in freeze-drying: I. Stability in glass-sealed rubber stoppered vials. *Developments in Industrial Microbiology* 26:397-405.

3. Barbaree, J.M., A. Sanchez and G.N. Sanden. 1985. Problems in freeze-drying: II. Cross-contamination during lyophilization. *Developments in Industrial Microbiology* 26:407-409.

4. Flink, J.M. and Knudsen, H. 1983. *An Introduction to Freeze Drying.* Strandberg Bogtryk/Offset, Denmark.

5. Flosdorf, E.W. 1949. *Freeze-Drying.* Reinhold Publishing Corporation, New York.

6. Greiff, D. 1971. Protein structure and freeze-drying: the effects of residual moisture and gases. *Cryobiology* 8:145-152.

7. Greiff, D. and W.A. Rightsel. 1965. An accelerated storage test for predicting the stability of suspensions of measles virus dried by sublimation in vacuum. *Journal of Immunology* 94:395-400.

8. Greaves, R.I.N., J. Nagington, and T.D. Kellaway. 1963. Preservation of living cells by freezing and by drying. *Federation Proceedings* 22:90-93.

9. Harris, R.J.C., Ed. 1954. *Biological Applications of Freezing and Drying.* Academic Press, New York.

10. Heckly, R.J. 1961. Preservation of bacteria by lyophilization. *Advances in Applied Microbiology* 3:1-76.

11. Heckly, R.J. 1985. Principles of preserving bacteria by freeze-drying. *Developments in Industrial Microbiology* 26:379-395.

12. King, C.J. 1971. *Freeze-Drying of Foods.* CRC Press, Cleveland.

13. May, M.C. E. Grim, R.M. Wheeler and J. West. 1982. Determination of residual moisture in freeze-dried viral vaccines: Karl Fischer, gravimetric, and thermogravimetric methodologies. *Journal of Biological Standardization* 10:249-259.

14. Mellor, J.D. 1978. *Fundamentals of Freeze-Drying.* Academic Press, London.

15. Nail, S.L. 1980. The effect of chamber pressure on heat transfer in the freeze-drying of parental solutions. *Journal of the Parental Drug Association* 34:358-368.

16. Nicholson, A.E. 1977. Predicting stability of lyophilized products. *Developments in Biological Standardization* 36:69-75.

17. Parkes, A.S., and A.U. Smith, Eds. 1960. *Recent Research in Freezing and Freeze-Drying.* Charles C. Thomas, Springfield.

18. Rey, L.R., Ed. 1960. *Traite de Lyophilization.* Hermann, Paris.

19. Rey, L.R., Ed. 1964. *Aspects Theorique et Industriels de la Lyophilisation.* Hermann, Paris.

20. Rowe, T.W.G. 1970. Freeze-drying of biological materials: some physical and engineering aspects. *Current Trends in Cryobiology:* 61-138.

21. Seligman, E.B. and J.F. Farber. 1971. Freeze-drying and residual moisture. *Cryobiology* 8:138-144.