Freeze Drying 101

Some helpful freeze drying basics
by Larry Ulfik

Lyophilization, commonly referred to as freeze drying, is the process of removing water from a product by sublimation, and desorption. This process is performed in lyophilization equipment which consists of a drying chamber with temperature controlled shelves, a condenser to trap water removed from the product, a cooling system to supply refrigerant to the shelves and condenser, and a vacuum system to reduce the pressure in the chamber and condenser to facilitate the drying process.

Lyophilizers can be supplied in a wide variety of sizes and configurations and can be equipped with options that allow system controls to range from fully manual to completely automatic. For pharmaceutical compounds that undergo hydraulic degradation, lyophilization offers a means of improving their stability and shelf life. Many parenteral medications such as vaccines, proteins, peptides, and antibiotics have been successfully lyophilized. New biotechnology products will also increase the demand for freeze-drying equipment and processes. Diagnostic products such as controls [Calibrators] and analyzers on a "chip" are a reality.

Early attempts at lyophilization were largely empirical in nature because the process variables were not thoroughly understood. However, much of the "black magic" of freeze-drying has been replaced through basic research over the last twenty years. Lyophilization equipment and control mechanisms continue to evolve, based on scientific evaluation of thermal, physical, and chemical data derived from freeze drying cycles and products. Process Analytical Technology has played an important role in this metamorphosis from an art to a science.

Lyophilization cycles consist of three phases: Freezing, primary drying, and secondary drying. Conditions in the dryer are varied through the cycle to insure that the resulting product has the desired physical and chemical properties, and that the required stability is achieved. Typically, products that are freeze dried have a shelf live exceeding 2 years.

During the freezing phase, the goal is to freeze the mobile water of the product. Significant super cooling may be encountered, so the product temperature may have to be much lower than the actual freezing point of the solution before freezing occurs. The rate of cooling will influence the structure of the frozen matrix. If the water freezes quickly, the ice crystals will be small. This may cause a finer pore structure in the product with higher resistance to flow of water vapor and longer primary drying time. If freezing is slower, ice crystals will grow from the cooling surface and may be larger. The resultant product may have coarser pore structure and perhaps a shorter primary drying time.

The method of cooling will also affect the structure and appearance of the matrix and final product. If the solution is frozen in vials on the cooled shelf, ice will grow from the bottom of the vial toward the top, while immersion in a cooling fluid will cause crystal growth from the bottom and sides of the vial. Because some materials form glassy layers, cooling conditions must be controlled to avoid the formation of the dense "skin" on the surface of the frozen product that may impede the escape of water vapor during subsequent drying phases. Methods have been developed to instantly freeze product on all shelves at the same time, or liquid nitrogen submersion equipment can be used either inside or outside the freeze dryer to accomplish a faster rate of freezing employing huge temperature difference afforded by liquid nitrogen.

A term that is frequently encountered in discussions about freeze-drying is eutectic point. On a phase diagram, this is the temperature and composition coordinate below which only the solid phase exists.

It should be understood that, depending on the composition of the solution, there might be more than one eutectic point for a product or none at all. During the freezing phase, the product may have to be cooled to a temperature below its lowest eutectic point. This temperature may then be maintained throughout the primary drying phase.

It should be noted that products do not necessarily have a eutectic point. For products with components that do not crystallize during freezing, drying should be performed at temperatures below the glass transition temperature of the amorphous phase (multi-component mixture). The glass transition temperature will be determined by the composition of the amorphous phase in the frozen product, which, in turn, is dictated by the product formulation and the freezing procedure employed. Mannitol and some other compounds can exist as an amorphous phase or exhibit a crystalline phase depending upon its thermal history. This is a very useful technique for drying product at higher rates using the crystalline form of the excipient. Other excipients may never exhibit a crystalline form and these may be instrumental in cryo-protecting sensitive molecules during freeze drying and even reconstitution.

In the primary drying phase, the chamber pressure is reduced, and heat is applied to the product to cause the frozen mobile water too sublime. The water vapor is collected on the surface of a condenser. The condenser must have sufficient surface area and cooling capacity to hold all of the sublimed water from the batch at a temperature lower than the product temperature. If the temperature of the ice on the condenser is warmer than the product, water vapor will tend to move toward the product, and drying will stop. One can describe freeze drying water vapor movement in terms of its moving from a place of higher pressure to a place of lower pressure. The water vapor moves at a very high rate of speed.

It is important to control the drying rate and the heating rate during this phase. If the drying proceeds too rapidly, the dried product can be blown out of the container by escaping water vapor. If the product is heated too rapidly, it will melt or collapse. This will cause degradation of the product, and will certainly change the physical characteristics of the dried material, making it visually unappealing and harder to reconstitute. While frozen mobile water is present, the product must be held below the eutectic temperature or glass transition temperature.

The components shown in Table A below cause products to have the indicated collapse temperatures.

As water sublimes, the product cools. Therefore, throughout this phase, the product will remain colder than the shelf temperature, which is supplying the heat of sublimation. At the end of primary drying, the product temperature will rise asymptotically toward the shelf temperature. This and several other methods may also be used to detect the endpoint of primary drying.

Many drying cycles use chamber pressure control, which in turn controls the drying rate. At very low pressure, the main form of heat transfer is conduction from the shelf through the bottom of the product container. Since glass is an insulator, this process is not very efficient, and drying can be slow. To improve the heat transfer mechanism, inert gas such as nitrogen may be introduced into the drying chamber at a controlled rate. The presence of these gas molecules facilitates heating of the walls of the container in addition to conduction through the bottom of the container, thereby increasing the amount of heat being supplied to the over time.. This will shorten the drying rate, reduce the cycle time, and reduce energy and labor costs associated with a lengthy process and it will raise product temperature. There are several ways to control chamber pressure depending upon the desired result.

However, if the pressure in the chamber exceeds the ice vapor pressure of the product, water may not be able to sublime. All of the energy from the heat source will be used to increase the product temperature until melting occurs. Therefore, the accuracy and precision of the pressure

control system are critical to successful lyophilization.

Since there is no mobile water in the product at the end of primary drying, the shelf temperature may be increased without causing melting. Therefore, shelf temperature is increased to drive off or desorb bound water such as water of crystallization until the residual water content falls to the range required for optimum product stability. This phase is referred to as secondary drying, and is usually performed at the maximum vacuum the dryer can achieve, although there are products that benefit from increased pressures, too. Be careful not to increase product temperatures too fast so as not to exceed the glass transition of some products. Products contain 10% or less water can still collapse if the tg[1] is exceeded.

Tg1: glass transition temperature

The product, its thermal history, and machine performance factors will determine the length of the secondary drying phase. Many products such as proteins and peptides require water to maintain secondary and tertiary structure. If this water is removed, the material may be denatured and lose some or all of its activity. In this case, water content must be carefully controlled. In addition, excessive heat may cause the dry cake to char or shrink.

Lyophilization equipment has improved over the years, and, with the advent of automated, sophisticated control mechanisms, has become much easier to use. The complexity of the controls, however, has made validation efforts more complicated and usually quite time consuming.

In addition to cooling, heating, and vacuum control functions already discussed, many freeze dryers will also incorporate clean in place (CIP), sterilize in place (SIP), computerized cycle control, and cycle monitoring functions. The reliable reproducible performance of all these functions must be validated rigorously to insure consistent product quality.

Citations for various compounds and mixtures are for general reference; data ignore important interactions, so use with this understanding.

Table A

Compound	t_c °C	Ref
Dextran	- 9	a
Sucrose (15%)	-22.5	b
PVP	-23	a
PVP	-17	b
Sucrose (45%)	-29	b
Lactose	-32	c
Inositol	-27	d
Sorbitol	-57	d
10% NaCl-KCl-H_2O	-24°C	e
5% NaCl-Mannitol-H_2O	-50°C (amorphous, Sorbitol, mannitol)	e
5% Mannitol, Sorbitol, H_2O	-34°C (amorphous, sorbitol, mannitol)	e
Lactose (1.3% residue moisture)	60°C glass transition of very dry lactose	f
Sucrose (.8% residual moisture)	60°C glass transition of very dry sucrose	f

Ficoll	-19°C	g
Ovalbumin	-10°C	g
PVP (K-30)	-23°C	g
Sephadex (G-200)	-10°C	g
Sucrose	-32°C	g
1.5% Sucrose, 3.5% Mannitol	-21C (Eutectic mannitol interaction with amorphous sucrose?)	g

a A.P. Mackenzie, Transplantation Proc., vil VIII, suppl. 1.181 (1976)

b R.J. Bellows and C.J. King, Cryobiology, 9, 559 (1972)

c M.J. Pikal, S. Shah, D. Senior, and J.E. Lang. J. Pharm. Sci., 72, 635 (1983)

d A.P. Mackenzie, Bull. Par. Drug Assoc., 20, 101 (1966)

e Chem. Pharm. Bull 19 (6) 1095-1102 (1971)

f J. Food Sce. 62 (4) 693-695 (1997)

g DHEW Publication (NIH-78-1422)

The Use of Solvents in Freeze Drying

There have been cases where solvents have been incorporated in freeze-drying formulations. Sometimes the incorporation is the result of a prior step in the manufacture of the product.

For example, pancreatin (porcine) is produced with up to 50% isopropanol by weight, in the final formulation. Blood is fractionated using cold alcohol, and sometimes in the final product there remains a residual of up to 10%.

Sometimes solvent is incorporated to enhance the solubility of one or more active ingredients. There seems to be more and more of this product type, especially in biotech products, and anti-cancer drugs. Some solvents utilized are acetonitrile, methanol, ethanol, propanol, tert-butyl alcohol, acetone cyclohexane, and DMSO.

It is important to understand that strictly speaking, it is very dangerous to use a non explosion-proof freeze dryer with most solvents.

The reason is Limits of inflammability.

Each of these compounds has a flammability range. For example, the compound acetonitrile is a colorless liquid that boils at 81°C, melts at -48°C, auto ignites at 973°C, has a flash point at 42°F, a vapor pressure of 72.8mm at 20°C (water has a vapor pressure of 760mm/hg at 100°C) a vapor density of 1.41 times heavier than air, and a density of .786, which means it is lighter than water. Its upper explosive level is 16% and its lower explosive level 4.4%. So, if the material is above 42°F and between 4.4% and 16% by weight mixed with air, the material can catch fire if exposed to a spark or flame.

The material also has some important health hazards that include: attack of the central nervous system, liver, and other organs. The time weighted average does over an 8-hour period is 20 PPM.

One can see that some of these solvents produce vapor pressures equivalent to water at lower temperatures. This volatile nature of solvents needs to be controlled in order to prevent fires. Acetonitrile water solutions are considered flammable down to the 15%-18% (w-w) level.

The Common Solvents chart is useful in another way. In an IPA water solution, isopropanol comes off first, creating a viscous fluid at the bottom of the condenser, which has a vapor pressure that is dependent on its temperature. The trick in freeze drying materials with a solvent such as this is to collect the solvent in a low temperature chilled "catchpot" connected to the condenser drain, and valve off the catchpot from the system at the appropriate time.

Pressure of materials at various temperatures:

Common Solvents

Compound Name	1mm	10mm	40mm	100mm	760mm	760mm	M.P.
Acetonitrile	-47	-16.3	7.7	27	62	81	-41
Methanol	-44	-16.2	5	21.2	49.9	64.7	-97.8
Isopropyl (2 propanol)	-26.1	2.4	23.8	39.5	67.8	82.5	-85.8
Water	-16	11.2	34	51	83	100	0
Acetic acid	-17.2	17.5	43	63	99	118	16.7
Benzyl alcohol	58	92.6	119.8	141.7	183	204.7	-15.3
Ethanol	-31.3	-2.3	19	34.9	63.5	78.4	-112
Tert-butyl alcohol	-20.4	5.5	24.5	39.8	68	82.9	25.3

It is sometimes important to control the pressure in the system with a valve connected to a pressure controller capable of 0-760mm/Hg ranges during this phase where liquid is collected. When the pressure is above setpoint, say at 1.5mm/Hg, the VBS opens allowing the vacuum pump to reduce the pressure below 1.5mm/Hg, whereupon the valve closes. In this way, one can keep the material from entering the vacuum pump by trapping it first in the condenser-catchpot arrangement.

Notice also that one could trap this material with a water-cooled condenser at 23.8°C if one were able to control the pressure at 40mm or 40000 microns/Hg.

Vacuum dryers utilize water-cooled condensers for this purpose.

At the end of the liquid collection cycle, the IPA will almost be gone, leaving mostly water remaining, whereupon the condenser has a low enough temperature to condense and solidify the remaining subliming vapors which are trapped at an appropriate temperature.

The lower temperature and lower pressure are needed because in order to keep the product temperature low, these conditions must be met. If one compares the vapor/temperature relationships between the solvent and water, solvent comes off at a low temperature at a very high pressure, whereas in order to maintain the low temperature in aqueous systems one must accomplish this at a lower temperature.