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Sterile Drug Products

Formulation, Packaging, Manufacturing, and Quality

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Baxter BioPharma Solutions
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Preface

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10 | Formulation of freeze-dried powders

With the advent of biotechnology medicines, freeze-drying formulation and process development have embarked on new heights of importance in the parenteral industry. Roughly 40% of commercial biopharmaceutical products are freeze-dried; this percentage likely will keep increasing with time. Freeze-drying and lyophilization mean the same thing. Freeze-drying perhaps is more accurate because the process involves both freezing of a solution and then removing the solvent from that solution that involves drying procedures. Lyophilization means to "love the dry state," but the title does not emphasize the cooling/freezing segment. Freeze-drying involves:

1. Compounding, filtering, and filling drug formulations as solutions into vials historically although now more syringes are being used as primary container for lyophilized products. Most of the discussion in this chapter will focus on the vial being the primary package.
2. Inserting a partially slotted rubber closure on the neck of the vial (Fig. 10-1) and transferring the containers into a freeze-drying chamber. If the vial, as well as syringes or cartridges, is to be part of a dual-chambered device (lyophilized powder in one compartment, diluent solution in the other compartment, separated by a rubber plunger), then no rubber closure is inserted prior to lyophilization.
3. Cooling the product to a predetermined temperature that assures that the solution in all containers in the freeze-dryer become frozen.
4. Adjusting the temperature of the shelf/shelves of the freeze-dryer that is as high as possible without causing the temperature of any product container to be above its "critical temperature" (eutectic temperature, glass transition temperature, collapse temperature).
5. Applying a predetermined vacuum that establishes the required pressure differential between the vapor pressure of the sublimation front of the product and the partial pressure of gas in the freeze-drying chamber that allows the removal of frozen ice from all product containers—the process of sublimation.
6. Increasing the shelf temperature once all the ice is sublimed in order to remove whatever remaining water is part of the solute composition to a residual moisture level predetermined to confer long-term stability of the drug product.
7. Completely inserting the rubber closure into the container via hydraulic-powered lowering of the dryer shelves.
8. Removing all freeze-dried containers, completing the sealing (or for syringes/cartridges adding the rubber septum), and carefully inspecting each product unit (inspection criteria for lyophilized products covered in chap. 22, Table 22-4).

This chapter will focus on the formulation of freeze-dried products, whereas chapter 20 will focus on the process of freeze-drying.

ADVANTAGES AND DISADVANTAGES OF FREEZE-DRYING

Freeze-drying is required for active pharmaceutical ingredients that are insufficiently stable in the solution state. Insufficiently stable means that the drug will excessively degrade in solution within a period of time not amendable to marketing the product as a ready-to-use solution. Many small and large molecules are labile in the presence of water and within several days to several weeks will degrade to a point that is unacceptable, usually more than 10% loss of activity or potency compared to the label claim amount of active ingredient. Were it not for freeze-drying technology, many important therapeutic agents would not be commercially available.

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Figure 10-1 Partially slotted stoppers in solution vials prior to loading into freeze-dryer.

Tables 10-1 and 10-2 present two lists of commercial freeze-dried products. Table 10-1 presents general information about these products, whereas Table 10-2 focuses more on the specific quantitative formulations for each product. They are not exhaustive and will not be up to date at the time of this publication, but provide excellent representative information about freeze-dried formulated products being successfully used to save and affect lives.

Besides overcoming stability problems by converting a solution to a dry powder, freeze-drying also offers the advantages of processing the product in the liquid form. Sterile powders can also be produced by other processes (not covered in this book) such as spray-drying, spray-freeze drying, or sterile crystallization followed by powder filling. However, freeze-drying offers certain advantages over other powder production processes including the fact that the product can be dried without the need for elevated temperatures, product sterility is more easily achieved and maintained, the contents of the dried material remain homogeneously dispersed, and the reconstitution times generally are faster. Also, for drugs that are oxygen sensitive, freeze-drying is a better powder-producing alternative, because the environment during the freeze-drying process can be an oxygen-free condition and an inert gas can fill the headspace of the container prior and during closing of the container.

Freeze-drying also has certain limitations, perhaps the foremost being cost compared to other powder-producing processes and certainly more expensive than liquid filling and stoppering. Volatile compounds in the formulation could be removed if high vacuum levels are required and high vacuum has been known to increase the extractable levels from the rubber closure. The freezing and drying steps are known to cause stability problems with some proteins that usually can be overcome using stabilizers called cryo- or lyoprotectants. Because the product has been previously sterilized prior to loading into the freeze-drying chamber, sterility must be maintained during the loading and unloading process and also during the freeze-drying process itself. The ability to maintain aseptic conditions during these processes as well as validating the sterilization of the freeze-dryer chamber and all connections and gases leading into the chamber must be demonstrated.

ATTRIBUTES AND REQUIREMENTS OF A FREEZE-DRIED PRODUCT

The ideal freeze-dried product has a very pleasing aesthetic appearance (i.e., intact cake, uniform color, and appearance) (Fig. 10-2), sufficient strength of active ingredient, chemical and physical stability, sufficient dryness and other specifications that are maintained throughout the product shelf-life, sufficient porosity that permits rapid reconstitution times, and freedom from microorganisms (sterility), pyrogens, and particulate matter after reconstitution. Also, after the drug is in solution, it must remain within certain predetermined specifications (e.g., potency,

(Text continues on page 154.)

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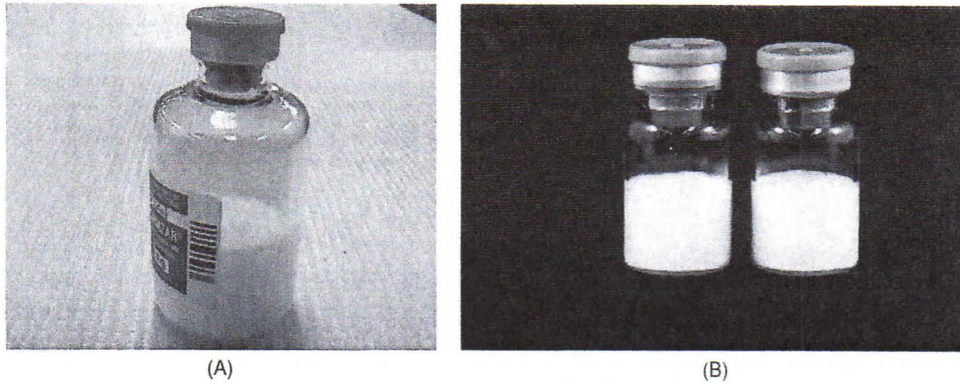

(A) (B)

Figure 10-2 Examples of a pharmaceutically elegant freeze-dry cakes. *Source:* Courtesies of Eli Lilly and Company (A) and Dr. Gregory Sacha, Baxter BioPharma Solutions (B).

pH, freedom from particulate matter) for a certain period of time prior to administration. The desired minimum time for solution stability after reconstitution is 24 hours at ambient temperature although many products, especially biopharmaceuticals, are insufficiently stable at ambient temperature and must be refrigerated even for these short periods of time. Also, European requirements that generally have been applied throughout the world require products without antimicrobial preservatives to be used (administered) "immediately," generally meaning within three hours after reconstitution. Freeze-dried products reconstituted with diluents containing antimicrobial preservatives can be stored for much longer times depending more on drug stability in solution than on potential microbial contamination concerns.

Freeze-dried formulation requirements usually are different depending on whether the active ingredient is a small molecule or large molecule. Formulation of a freeze-dried product containing a small molecule often does not need any additives, depending on the amount of active ingredient per container. For example, many freeze-dried antibiotic products contain only the antibiotic. If the active constituent of the freeze-dried products is present in a small quantity (usually less than 100 mg) where, if freeze-dried alone, its presence would be hard to detect visually, then additives are used. This is true for many small-molecule freeze-dried products, for example, those containing anticancer agents, and practically always true for large-molecule freeze-dried products. The solid content of the original product ideally should be between 5% and 30%. Therefore, excipients often are added to increase the amount of solids. Such excipients are called "bulking agents"; the most commonly used bulking agent in freeze-dried formulations is mannitol. However, most freeze-dried formulations must contain other excipients because of the need to buffer the product and/or to protect the active ingredient from the adverse effects of freezing and/or drying. Thus, buffering agents such as sodium or potassium phosphate, sodium acetate, and sodium citrate are commonly used in freeze-dried formulations. Sucrose, trehalose, dextran, and amino acids such as glycine are commonly used lyoprotectants. Other types of stabilizing excipients often required in freeze-dried formulations are surface-active agents or competitive binding agents. Other reasons for adding excipients freeze-dried compositions, although typically these are part of the diluent formulation rather than the freeze-dried formulation, are tonicity-adjusting agents and antimicrobial preservatives for multiple-dose applications.

Each of these substances contribute to the appearance characteristic of the finished dry product (plug), such as whether the appearance of the finished product is dull and spongy or sparkling and crystalline, firm or friable, expanded or shrunken, or uniform or striated. Therefore, the formulation of a product to be freeze-dried must include consideration not only of the nature and stability characteristics required during the liquid state, both freshly prepared and when reconstituted before use, but also the characteristics desired in the dried product as it is released for commercial use and distributed to the ultimate user.

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A "rule-of-thumb" for freeze-dried products containing small molecules is "the drier, the better" because most stability problems with small molecules are moisture-related. However, for freeze-dried products containing large molecules, "drier is not necessarily better." Each molecule is different, but in general for large molecules, the effects of freezing and drying may be as much or more deleterious to the active constituent as the potential for hydrolytic degradation.

FORMULATION COMPONENTS IN FREEZE-DRIED PRODUCTS

Freeze-dried drug molecules, evidenced by the requirement to be freeze-dried, are relatively unstable molecules. Even in the dry state, freeze-dried formulations typically require additives for maintaining pH, isotonicity, or protection against adverse effects of the freezing and/or drying process. Additives may also be required, not for dry-state purposes, but to maintain stability and, in some cases, solubility of the drug in solution after adding a reconstitution diluent. Such additives to enhance solution stability and solubility include buffers, surface-active agents, and complexing agents. For drugs reconstituted to serve as multiple-dose products, an antimicrobial preservative system must be part of the freeze-dried formulation or part of the reconstitution diluent. Table 10-3 lists examples of formulation additives in freeze-dried formulations.

Freeze-dried formulations containing small molecules either do not require any additive excipients because of the large quantity of drug to be freeze-dried, (typically more than 100 mg per container), or additives required are for relatively simple purposes such as adding bulk to the powder, buffering the formulation, providing isotonicity, or perhaps helping to maintain solubility of the drug. Formulation challenges for small molecule formulations are relatively simple at least for the experienced formulation scientist.

Stabilizing large molecules during freeze-drying requires much more formulation expertise and challenge. Freeze-dried formulations of large molecules typically contain one or more of the following additives: bulking agents, lyoprotectants, surfactants, and buffers. Some large-molecule freeze-dried formulations, typically when the protein content is so dilute (low mg to ng/mL levels), contain human serum albumin or some other component to serve as competitive binders to minimize loss of protein due to adsorption to manufacturing surfaces (filters, tubing, disposable mixing bags, stainless steel) and primary container surfaces (glass and rubber). Certain additives such as mannitol and sucrose also may serve as tonicity modifiers. Salts usually are avoided because they decrease the critical temperature of the formulation (lower eutectic or glass transition temperature) and are known to cause concentration-dependent destabilization effects on proteins. Table 10-2 presents a listing of freeze-dried protein formulations, not to be exhaustive but to give the reader an idea of the qualitative composition of these formulations.

Some protein molecules can be adversely affected by the freeze-drying process, that is, the process of freezing and/or drying can cause the protein to denature and aggregate and lose potency. Certain excipient stabilizers have been found to minimize or prevent the problems caused by freezing and/or drying. Excipients that stabilize the protein against the effects of freezing are called cryoprotectants. The primary theory, although not completely accepted,

Table 10-3 Additive Categories and Examples for Freeze-Dried Formulations

Category	Example(s)
Bulking agents	Mannitol, lactose, glycine
Stabilizers	
"Ridigizers" (prevent collapse)	Mannitol, glycine
Minimize aggregation	Polysorbate 20 or 80; poloxamer 188
Cryoprotection	Polyethylene glycol, some sugars
Lyoprotection	Sucrose, trehalose
Minimize surface adsorption	Human serum albumin, polysorbates
Buffers	Acetate, citrate, phosphate, Tris, amino acids
Collapse temperature modifiers	Dextran, polyethylene glycol, disaccharide sugars
Tonicity modifiers	Mannitol, sodium chloride, glycerin

for explaining the cryoprotective effects of certain additives, is called the "excluded solute" or "preferential exclusion" theory (1–3). Some scientists have suggested that solutes that help protect the protein from dissociating during freezing do so because they are excluded from the surface of the protein, as can be demonstrated by dialysis experiments (where the protein and the excipient are not found together in the dialysate). When solutes are excluded from the protein surface, the chemical potential of both the protein and the solute increase. This presents a thermodynamically unfavorable environment for the denatured form of the protein as the denatured form is an unfolded form and yields a greater surface area to the solvent. The native form, with less surface area, is therefore thermodynamically favored.

Another way of explaining the effects of cryoprotectants is the fact that they induce preferential hydration of the surface of the protein because by not binding at the protein surface, this favors water molecules to bind preferentially and this helps to stabilize the native protein state.

Sugars (sucrose, lactose, glucose, trehalose), polyols (glycerol, mannitol, sorbitol), amino acids (glycine, alanine, lysine), and polymers (polyethylene glycol, dextran, polyvinylpyrrolidone) all serve as potential cryoprotectants. The best or, at least, most preferred cryoprotectants appear to be polyethylene glycol (PEG) (molecular weight 3350 Daltons), sucrose, and trehalose.

For proteins requiring both cryo- and lyoprotection, it may be judicious to employ both an agent such as PEG along with a sugar. An example of a marketed therapeutic with this combination is Venoglobulin-S, which contains PEG and sorbitol. A potential caveat to using PEG in lyophilized formulations is the possibility of a liquid–liquid phase separation induced by freeze-concentration, an event implicated in protein unfolding (4).

Proteins may not denature or experience any loss of potency during freezing or in the frozen state, but may experience adverse effects when the sublimation process occurs and when stored in the dry state. Such proteins need stabilizers called lyoprotectants. Lyoprotectants appear to stabilize proteins from the effects of drying and the dry state by what is referred to as the "water-substitute" hypothesis or the "vitrification" hypothesis. Sugars are excellent lyoprotectants. They provide a glassy matrix that retards molecular motions and reduces the rates of deleterious reactions (5,6). They also decrease protein–protein contacts and inhibit deleterious reactions depending on such contacts (e.g., aggregation) (7–9). Sugars serve as water-replacement substrates that form hydrogen bonds to proteins in the dried state (4). The water-replacement or substitute hypothesis is supported by solid-state studies exploring techniques such as Fourier-transform infrared (10), water sorption (11,12), and dissolution calorimetry (13). It is likely sugars have all these possible mechanistic roles in their ability to stabilize proteins.

Often the same excipient can provide both cryo- and/or lyoprotection. An example of cryoprotection is the stabilization effect of sucrose, trehalose, sorbitol, and gelatin on a recombinant adenoviral preparation (14). An example of lyoprotection is the stabilizing effect of lactose and other sugars on recombinant human growth hormone (rhGH) (15). However, lactose, a reducing sugar, is not preferred because of its potential adduct formation.

In both dry state theories, it is important that the excipient stabilizer, the lyoprotectant, exist in the amorphous state, hence the name "vitrification" (glass formation). Protein stability in the dry state results from the protein existing with an amorphous solute in an inert, rigid amorphic matrix where the water content in the matrix also helps to stabilize the protein. Obviously, too much excess water and the protein will degrade by chemical processes (e.g., deamidation), but proteins need a certain amount of water to maintain secondary and higher structure. Thus, excipients that remain amorphous during the freeze-dry process molecularly interact with the amorphous protein and together the matrix confers stability on the protein for long-term stability in the dry state. It has been shown that, for optimal stabilization, the sugar excipient should remain in the same amorphous phase containing the peptide or protein (all of the above mechanisms are consistent with this observation). For example, crystallization of mannitol has been implicated to explain incomplete stabilization of lyophilized rhGH (16) and the structure of bovine serum albumin, ovalbumin, β-lactoglobulin, and lactate dehydrogenase (LDH) upon freeze-drying (17). In addition to crystallization, separation of amorphous phases can also occur, particularly in the frozen state.

Once excipients crystallize, they no longer molecularly interact with the protein and cannot protect it. Amorphous excipients, combined with the protein, have a unique glass transition

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temperature (T_g) in the dry state. If storage temperature exceeds the glass transition temperature, the physical state of the dried matrix changes from a glassy solid to a rubbery solid that can result in collapse or partial collapse of the freeze-dried cake. Product collapse is not necessarily detrimental to the stability of some proteins, although pharmaceutical elegance still remains an important quality parameter of freeze-dried products.

Gradual conversion of excipients from the amorphous to the crystalline state occurs when there is adequate molecular mobility for nucleation and crystal growth (12). Molecular mobility is a term used to describe the movement of molecules in a formulation. Water will act as a plasticizer to lower the glass transition temperature of amorphous solids and increase the molecular mobility of the amorphous system (18). Molecular mobility typically occurs when the amorphous solid is stored at a temperature greater than its glass transition temperature, but can also occur at temperatures below the glass transition temperature of certain amorphous solids (19,20). Molecular mobility of protein molecules can be measured by solid-state ^{1}H nuclear magnetic resonance (21), nuclear magnetic resonance relaxation based critical mobility temperature (22), and ^{13}C solid-state nuclear magnetic resonance (23). All these techniques measure water mobility in lyophilized formulations and this can be correlated to protein stability.

Additives in a formulation can prevent crystallization of carbohydrates. Examples include high molecular weight polymers (e.g., dextran and polyvinylpyrrolidone, (24) and proteins (e.g., recombinant bovine somatotropin (BST) (12). Polymers can increase the glass transition temperature, thereby decreasing the mobility of the amorphous solute, whereas proteins such as BST are thought to interfere with either nucleation rates or number of nuclei formed to support a crystal.

Alpha$_1$-antitrypsin (rAAT) is an example of a recombinant protein that must be freeze-dried, yet does not need cryo- or lyoprotection (25). It is interesting that some proteins use cryo-and/or lyoprotectants while others do not (Table 10-2). Formulations without cryo-and/or lyoprotectants either truly are sufficiently stable (e.g., alteplase, α-1 proteinase inhibitor, glucagon, human chorionic gonadotropin) without the need for these stabilizers or may not be all that stable and must be refrigerated in the solid state (e.g., aldesleukin, asparaginase).

CONCENTRATIONS OF STABILIZERS

If cryoprotection is needed, the relevant concentration of the stabilizer in solution prior to freezing should be on the order of 0.3 M or above. If lyoprotection is required, the relevant concentration depends on the level of protein present. The sugar stabilizer needs to be in the proper ratio to the protein (either mole ratio to satisfy water "binding" sites or volume ratio if the relevant degradation mechanism(s) relate directly to glass dynamics and dilution of the protein in an inert solid matrix). In practice, a mass ratio of about 1:1 (sugar:protein) is usually needed for proper stabilization, regardless of the mechanism(s) that might be operating.

There are some data to suggest that the amount of sugar for optimum protection should be enough to satisfy sites on the dried protein that have a strong affinity for water (e.g., charged or polar residues). Studies of water vapor sorption on solid proteins describe this level as a "water monolayer" (11,26). Satisfying these sites (by providing enough amorphous sugars to interact with them in the dried state) stabilizes proteins. For instance, rhGH contains approximately sixty-six strongly water binding sites per molecule protein; about this amount of various sugars was required for maximum stabilization of the lyophilizate upon accelerated storage (16). For recombinant humanized monoclonal antibody (rhuMAb), a much larger molecule containing approximately 500 such strongly water-binding sites, a ratio of about 360 to 500 moles of sugar per mole protein afforded the best storage stability for freeze-dried protein (27). Formulations with combinations of sucrose (20 mM) or trehalose (20 mM) and mannitol (40 mM) had comparable stability to those with sucrose or trehalose alone at 60 mM. Formulations with mannitol alone were less stable.

In order to provide protection in the dried state, a stabilizing sugar should generally remain amorphous in the same phase as the protein. Even so, crystallizing sugars (e.g., mannitol) are widely used, either as a bulking agent or to promote stability. In this regard, a combination of amorphous and crystallizing stabilizing excipients may be employed. In this case, the presence of the amorphous agent could serve to retard crystallization of the other. For example, it was reported for lyophilized rhuMAb that a combination of sucrose and mannitol provided

stabilization, provided that the total amount of sugar satisfied the level cited above (28). ENBREL provides an example of a marketed biopharmaceutical product in a freeze-dried form and containing a combination of mannitol and sucrose.

In addition to sugars, proteins such as gelatin and albumin are also employed to provide general stabilization in lyophilized biopharmaceutical products. In particular, human albumin (purified from plasma) is widely found in biopharmaceuticals (and, by itself, is also considered a biopharmaceutical product).

CRYSTALLINE AND AMORPHOUS EXCIPIENTS

In general, a freeze-dried formulation that is predominately crystalline will "look" better, that is, look more pharmaceutically elegant than a formulation that is predominately amorphous. Crystalline solutes are also much easier to dry because the water is adsorbed on the surface of the solute rather than within the molecular structure of the solute, as is the case with amorphous solutes. However, amorphous formulations offer stability advantages if the active ingredient is a protein, minimize the potential for overdrying of the product, and can adsorb moisture that over time may be released from the rubber closure.

Moisture content of a freeze-dried product is obviously an important property to monitor. In general, too much moisture means that the product will eventually collapse and the active ingredient will degrade chemically. Each freeze-dried product must be studied for the moisture specifications required for long-term stability in the dry state. Residual moisture specifications for most products fall within the range of 0.5% to 3.0%. The amount of residual moisture is not as important as where the water resides in the freeze-dry matrix and what kind of solid morphology exists. If the matrix is crystalline, water exists as surface water and is not likely problematic. However, if the matrix is amorphous, or if the active is amorphous with the rest of the matrix crystalline, then excess water may interact molecularly with the drug and cause unacceptable degradation.

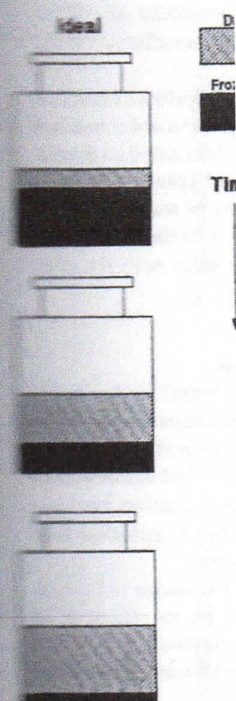
The judicious use of excipients can greatly influence product stability. Methylprednisolone was freeze-dried in the presence of mannitol or lactose (29). Although moisture content in the two cakes was identical, the rate of hydrolysis was higher when mannitol was the bulking agent. Mannitol crystallized during freeze-drying and had little to no interaction with water in the microenvironment of the drug. In fact, crystallized mannitol is essentially anhydrous, and any residual water will localize in the amorphous drug phase only. Lactose, however, did not crystallize and served to interact with residual water, thus preventing it from interacting with and hydrolyzing the drug. The degree of crystallinity of the bulking agent can have significant effect on the distribution of water in the freeze-dried matrix.

Distribution of residual moisture in the finished dried product is as important as the overall water content. Moisture content was measured immediately after freeze-drying in three sections of a lyophilized product (top, middle, and bottom of the plug) as well as moisture along the vial wall (30). They found that moisture content in the top section was less than moisture content in the bottom section and that the lowest moisture content of the entire plug existed along the walls in the vial (Fig. 10-3). Thus, drying along the vial walls occurs faster than drying in the plug core. They proposed that faster drying along the vial walls is a result of observed product shrinkage during drying, providing a low resistance pathway for vapor escape along the vial wall.

Many freeze-dried formulations contain three solid components—the active, a crystalline bulking agent, and an amorphous stabilizer. A good example is the formulation for human growth hormone where both mannitol and glycine are additives with mannitol crystallizing during freeze-drying and glycine remaining amorphous. A review of the formulations listed in Table 10-2 shows that several contain more than one bulking agent/stabilizer that helps to maintain an amorphous component and form a protective amorphic matrix with the protein.

MANNITOL

Mannitol, being a major excipient used in lyophilized formulations, is the subject of many papers. Mannitol crystallization is highly influenced by freezing rate (31–33), concentration (32), and other excipients present in the formulation such as sucrose, trehalose, citric acid, hydroxypropyl-β-cyclodextrin, polysorbate 80 (34) and phosphate buffers and polymers (33).



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ell as moisture along
s less than moisture
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a result of observed
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active, a crystalline
nulation for human
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formulations listed
bilizer that helps to
with the protein.

he subject of many
-33), concentration
ehalose, citric acid,
and polymers (33).

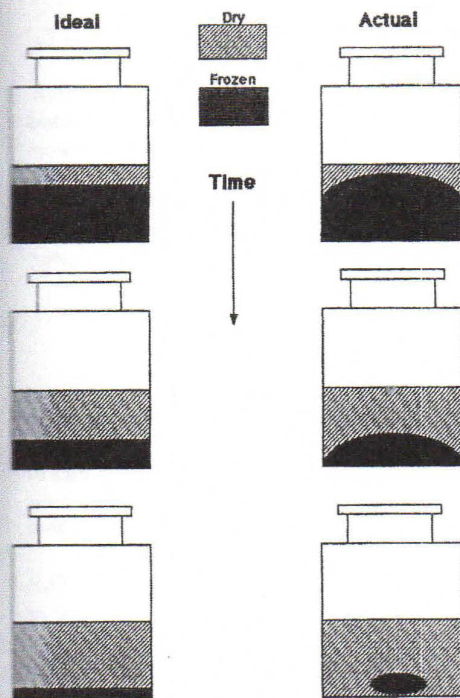


Figure 10-3 Schematic of suggested geometry of the ice–vapor interface during primary drying: A comparison of the "ideal" planar geometry with the curved interface geometry proposed. *Source*: From Ref. 30.

Sucrose especially will affect degree of mannitol crystallization and cause higher levels of mannitol hydrate and resultant residual moisture (35).

Mannitol crystallizes into different polymorphic forms as a function of concentration (relative to other components in the formulation) and freezing rate. Low concentrations of mannitol formed the δ polymorph while higher concentrations favored the formation of the β polymorph (36). At least three polymorphs of mannitol are present at different ratios in the lyophilized product depending on the freezing rate. Rapid freezing produces the α polymorph predominantly while slower rates (0.5°C/min) favor the formation of the δ polymorph. Annealing the frozen product will result in the β polymorph being most prominent. One-year storage will cause the δ polymorph to convert to a combination of α and β polymorphs. There is no evidence that the formation of the α, β, or γ polymorphic forms of mannitol, alone or in various combinations, has any effect on drying/processing characteristics, cake appearance, and or product stability.

A hydrate of mannitol can form during freeze-drying, particularly in conditions not conducive for producing well-developed crystalline mannitol, for example, low temperatures and concentrated solutions (37). This seems impossible, but the authors show thermal and crystallographic evidence for a hydrated form of mannitol that survives a freeze-dry cycle. This hydrate is metastable, able to convert to anhydrous polymorphs of mannitol upon heating. While not specifically investigated, these authors theorized that mannitol hydrates can potentially reduce the drying rates of mannitol-containing formulations and can redistribute residual water to the drug substance upon mannitol crystallization during storage at accelerated conditions. Because mannitol hydrate formation varied greatly from vial to vial, even in the same batch, this also potentially could lead to problems with vial-to-vial variation in moisture levels. Annealing during the freezing stage is the best approach to promote crystallization of the anhydrous form and reduce or eliminate the mannitol hydrate.

The formation of mannitol hydrate formed during freezing may be desolvated and converted to the anhydrous form by conducting secondary drying at 40°C or higher (38). In this paper it was also emphasized that mixtures of mannitol and sucrose in a 4 to 1 ratio successfully produced stable lyophilized formulations of four proteins (daniplestim, leridistim,

promegapoietin, and progenipoietin) using a primary drying product temperature of −10°C. The crystalline mannitol allows primary drying to be performed at temperatures above the T_g' of amorphous sucrose in the formulation.

Maintaining mannitol in the amorphous state during freeze-drying for optimal stabilization can be accomplished (39). All the enzymes studied were protected when mannitol remained amorphous, but become unstable with an increase in mannitol crystallinity. Mannitol in freeze-dried cakes containing enzyme and sodium phosphate buffer remained amorphous at lower concentrations (< 200 mM), although annealing the frozen solution resulted in mannitol crystallization. However, mannitol at higher concentrations (> 250 mM) in this enzyme-phosphate formulation crystallized and had no protective effects on preserving enzyme activity after freeze-drying.

MORE ON STABILIZING EXCIPIENTS IN LYOPHILIZED FORMULATIONS

Plasticizers (examples include glycerol, propylene glycol, ethylene glycol, or DMSO) will modify disaccharide and polymeric lyoprotective glasses (40). The proposed mechanism of protein stabilization was attributed to the following: the plasticizers fill small volumes left open by the larger (or stiffer) host glass-former, restricting motion, and thereby slowing the fast dynamics of the glass and subsequent protein degradation. This approach has narrow application because of the relatively large amounts of plasticizer required, and also because of the suggestion that the lower molecular weight oligomers like ethylene glycol are better stabilizers.

Raffinose will not lyoprotect an unstable drug as well as sucrose or trehalose (41). This observation was in contrast to other studies (42–44) showing raffinose to be as effective as trehalose and superior to lactose, maltose, and sucrose in stabilizing several enzymes. A possible explanation for these differences may involve differences in dehydration conditions and storage temperatures.

Mannitol and glycine in frozen solutions will influence the crystallization of each other (45). Glycine was shown to have a stronger initial tendency to crystallize, while it was easier to influence the crystallization of mannitol. Buffer salts, such as sodium phosphate, inhibited crystallization of both mannitol and glycine. The activity of LDH correlated with the extent of crystallization of these excipients (Fig. 10-4).

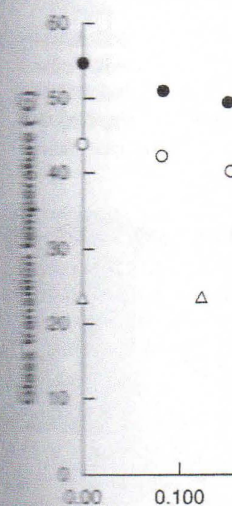
LDH formulations containing maltodextrin protected LDH against inactivation during freeze-drying because of the amorphous nature of these partially hydrolyzed starches (46). Maltodextrins were also reported to be better lyoprotectants for LDH than sucrose or maltose, although the mechanism is unknown.

The stabilization effects of amorphous additives on freeze-dried proteins are well accepted on the basis of several publications. β-Galactosidase was stabilized with inositol as long as this excipient stayed amorphous, but if inositol crystallized during storage, the enzyme activity declined (47). Inositol crystallization was prevented by addition of polymers such as dextran, Ficoll, and sodium carboxymethylcellulose.

Stabilization of lyophilized proteins not only depends on the formulation and dehydration process parameters, but also depends sometimes on the formulation of the reconstitution medium (48). Keratinocyte growth factor aggregation upon reconstitution with water can occur readily, but several additives such as sulfated polysaccharides, surfactants, polyphosphates, and amino acids in the reconstitution medium will significantly reduce this aggregation.

The Gordon–Taylor equation: $$T_g = \frac{[W_a T_{ga} + k(1 - W_a)T_{gb}]}{[W_a + k(1 - W_a)]}$$

was applied to predict the glass transition temperature of a model tripeptide in the presence of different sugars (sucrose, lactose, trehalose, and maltose) in both frozen solutions and in lyophilized products (49). Correlation was excellent between the predicted and actual T_g for various ratios of tripeptide to sugars. The authors also showed a significant effect of sodium chloride on the T_g' of the tripeptide in frozen solution, but no effect after lyophilization. Figure 10-5 from this paper demonstrates the plasticizing effect of water on decreasing the glass transition temperature.



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