Review

Design of Freeze-Drying Processes for Pharmaceuticals: Practical Advice

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Design of freeze-drying processes is often approached with a "trial and error" experimental plan or, worse yet, the protocol used in the first laboratory run is adopted without further attempts at optimization. Consequently, commercial freeze-drying processes are often neither robust nor efficient. It is our thesis that design of an "optimized" freeze-drying process is not particularly difficult for most products, as long as some simple rules based on well-accepted scientific principles are followed. It is the purpose of this review to discuss the scientific foundations of the freeze-drying process design and then to consolidate these principles into a set of guidelines for rational process design and optimization. General advice is given concerning common stability issues with proteins, but unusual and difficult stability issues are beyond the scope of this review. Control of ice nucleation and crystallization during the freezing step is discussed, and the impact of freezing on the rest of the process and final product quality is reviewed. Representative freezing protocols are presented. The significance of the collapse temperature and the thermal transition, denoted T_g', are discussed, and procedures for the selection of the "target product temperature" for primary drying are presented. Furthermore, guidelines are given for selection of the optimal shelf temperature and chamber pressure settings required to achieve the target product temperature without thermal and/or mass transfer overload of the freeze dryer. Finally, guidelines and "rules" for optimization of secondary drying and representative secondary drying protocols are presented.

KEY WORDS: lyophilization; freeze drying; secondary drying; formulation.

INTRODUCTION

Freeze drying, also known as lyophilization, is widely used for pharmaceuticals to improve the stability and long-term storage stability of labile drugs, especially protein drugs (1,2). Freeze-dried formulations not only have the advantage of better stability, but also provide easy handling (shipping and storage) (3,4). Freeze drying is a time- and energy-intensive process that could take days or even weeks to finish if the freeze-drying cycle is not optimized (1,5–7). The stability of the drug during freeze drying and storage (8) and the duration of the cycle are the two major considerations for freeze-drying process optimization. Given a freeze-drying process yielding a stable product, the shorter freeze-drying cycle has the advantage of higher throughput for a given dryer, and therefore a plant may use fewer freeze dryers for a given amount of product. A nonoptimum freeze-drying process may compromise drug stability, take longer, and cost more than is necessary.

In order to design an optimum freeze-drying process, process development scientists need to know the critical properties of the formulation and how to apply this information to process design. The critical formulation properties include the collapse temperature of the formulation, the stability of the drug, and the properties of the excipients used. The macroscopic collapse temperature of the formulation (T_c) is the temperature above which the freeze-dried product loses macroscopic structure and collapses during freeze drying (9). T_c is usually about 2°C higher than T_g', which is often associated with the glass transition temperature in the frozen state (10), or equals the eutectic temperature (T_{eu}) if solutes are crystallized in the frozen solution. In a mixture of crystalline and amorphous freeze concentrate where the crystalline phase is in excess, the amorphous phase collapses if the product temperature is above T_g' (or collapse temperature of amorphous phase), but gross or "macroscopic" collapse will not occur unless the product temperature is above both T_g' and T_{eu}. Between T_g' and T_{eu}, the amorphous phase collapses onto the surface of crystalline phase, and the crystalline phase provides mechanical support (11). Therefore, in a mixture of amorphous and crystalline phases dominated by crystalline phase, the T_c is close to T_{eu}. In order to produce an acceptable freeze-dried product, it is always required to freeze dry a formulation at the temperature lower than T_c (1,2).

The stability issue is particularly important for protein drugs. Proteins are sensitive to the stresses imposed by freeze drying and are easily degraded or decomposed during the process. The low temperature of freeze drying does not guarantee protein stability because many proteins experience cold denaturation or denaturation at interfaces (protein–air and

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protein–ice) (12–15). Fortunately, thermodynamic instability caused by low temperature or other stresses does not necessarily mean unfolding during freeze drying if the rate of unfolding is sufficiently slow on the time scale of the process so that drying is over before significant unfolding can occur. If so, this means that the protein might safely be freeze-dried at a temperature far higher than T_g', allowing a much faster drying process. This issue is discussed in more detail later in this review.

In general, the optimum freeze-drying process is that which achieves the highest drug quality for the least cost, which requires optimization of all the controllable stages of freeze drying. This review is intended to serve as a guideline to rational process design and optimization.

THREE STAGES OF FREEZE DRYING

A typical freeze-drying process consists of three stages; that is, freezing, primary drying, and secondary drying. Freezing is an efficient dessication step where most of the solvent, typically water, is separated from the solutes to form ice. As freezing progresses, the solute phase becomes highly concentrated and is termed the "freeze concentrate." By the end of freezing, the freeze concentrate usually contains only about 20% of water (w/w), or less than 1% of total water in the solution before ice formation. The freezing stage typically takes several hours to finish. Primary drying, or ice sublimation, begins whenever the chamber pressure is reduced and the shelf temperature is raised to supply the heat removed by ice sublimation. During primary drying, the chamber pressure is well below the vapor pressure of ice, and ice is transferred from the product to the condenser by sublimation and crystallization onto the cold coils/plates ($<-50°C$) in the condenser. Typically, the primary drying stage is the longest stage of freeze drying and optimization of this stage has a large impact on process economics. Secondary drying is the stage where water is desorbed from the freeze concentrate, usually at elevated temperature and low pressure. Some secondary drying occurs even at the very beginning of primary drying as ice is removed from a region, but the bulk of secondary drying occurs after primary drying is over and the product temperature has increased. Secondary drying normally takes only hours, and the opportunity for time reduction by process optimization is limited (16). With an optimum freeze-drying process, the freeze-drying process is optimized for all the three stages.

Freezing

Freezing is the first stage of freeze drying and is the stage where most of the water is removed from drug and excipients, the system separates into multiple phases, and the interfaces between ice and drug phase form. Freezing often induces many destabilizing stresses, particularly for protein drugs. These stresses include increase of protein concentration that enhances the protein–protein interaction leading to aggregation, pH change arising from crystallization of buffer salts, reduced hydrophobic interactions caused by the "dehydration" effect of ice formation that removes bulk water from the protein phase, formation of large ice–aqueous interfaces, and an enormous increase in ionic strength (16). The introduction of the ice–aqueous interfaces and pH shifts are well-known to

cause protein stability problems. The pH shift during freezing can be minimized by optimal choice of buffer salts (i.e., avoid phosphate, succinate, and tartrate) or by reducing buffer concentration to several mM (17–19). Protein degradation at the ice–aqueous interface can be minimized by increasing protein concentration (i.e., "saturate" the protein–ice interface) and/or by using surfactants (14). For a given protein formulation, process design also plays a very important role in protein stabilization.

Cooling Rate

One practical process approach to stabilization is to minimize the surface area of ice by growing large ice crystals which can be achieved by reduced supercooling. The degree of supercooling is the temperature difference between the thermodynamic or equilibrium ice formation temperature and the actual temperature at which ice begins to form, which is usually around 10 to 25°C lower but changes with cooling rate and other factors. Higher supercooling results in more/smaller ice crystals and larger ice specific surface area. Different freezing methods, like liquid nitrogen freezing, loading vials onto precooled shelves, or ramped cooling on the shelves, give different supercooling effects with normally the highest supercooling with liquid nitrogen freezing of small volumes and the lowest supercooling for the precooled shelf method (20). It was reported that slow cooling (0.5°C/min) caused larger supercooling effects than the precooled shelf method (20). However, the precooled shelf method gave large heterogeneity in supercooling between vials, which is undesirable (20). Normally, it is not practical to manipulate the supercooling by changing the cooling rate in a freeze dryer because the cooling rates are usually limited to less than 2°C/min, and the degree of supercooling is unlikely to change within such a small range (21). Slow freezing has the potential to increase the protein damage in systems prone to phase separation because phase separation is a kinetic process and provides enough time for the process to occur (22). If phase separation causes separation of protein and stabilizer, the stabilization effect will be lost. Phase separation is most common when polymers are used for stabilizers (23,24). Slow freezing also prolongs the time the protein exists in a concentrated fluid state where biomolecular degradation reactions are accelerated. We find a moderate cooling rate (about 1°C/min) is a good compromise. A cooling rate of this magnitude yields moderate supercooling with moderate ice surface area and a reasonably fast freezing rate, which is generally best for both those formulations prone to phase separation and those where phase separation is not an issue. It also (usually) produces uniform ice structure both within a given vial and from vial-to-vial.

Freezing Temperature and Time

After freezing, the formulation should be in solid state; that is, the drug phase should be below T_g' if amorphous or below T_{eu} if it is in the crystalline state. This condition requires the shelf temperature for freezing be set below T_g' or T_{eu}, and the product batch must be kept at the temperature long enough such that all solution has transformed into solid. Because of the limited thermal conductivity between vials and shelf, complete freezing requires significant time. The freez-

ing time depends on fill volume; that is, the larger fill volume takes longer to fully freeze (1). Generally, we use a final shelf temperature of $-40°C$ if the T_g' or T_{eu} is higher than $-38°C$; otherwise, we use a temperature $2°C$ less than T_g' or T_{eu} and allow time for complete freezing. The final temperature is held for 1 h if the fill depth is less than or equal to1 cm or 2 h if the fill depth is greater than 1 cm. Fill depth greater than 2 cm should be avoided when possible, but if necessary, freezing time should be increased proportionately.

Annealing

Aneaing is simply holding the product at a temperature above the final freezing temperature for a defined period to crystallize the potentially crystalline components (usually, crystalline bulking agent) in the formulation during the freezing stage. An annealing step is frequently necessary to allow efficient crystallization of the crystalline bulking agent, such as mannitol or glycine. Failure to crystallize the bulking agent has the potential of depressing the T_g' and compromising storage stability by crystallizing from the solid during storage (25). If the bulking agent crystallizes during primary drying, vial breakage may result, which is common if a high fill depth of concentrated mannitol is used (26). Vial breakage can be prevented by crystallization of mannitol during freezing using slow freezing or by avoiding a temperature lower than about $-25°C$ until the mannitol has completely crystallized. Completion of crystallization may be facilitated by annealing. The annealing temperature should be between the T_g' of amorphous phase and T_{eu} of bulking agent to give a high crystallization rate and complete crystallization. Sufficient annealing time is needed for completion of crystallization. The optimum time depends on the ratio and properties of the bulking agent used. A high mass ratio of bulking agent to other solutes (>80% of total solute, recommended) crystallizes much faster than a lower ratio (<50% of total solute, not recommended) (Tang and Pikal, unpublished). A low annealing temperature may tend to produce high crystallinity because supersaturation is higher at low temperature, but the crystallization rate may be too low becuse of high viscosity. The optimum annealing conditions are a compromise between crystallinity and crystallization rate. For mannitol or glycine, a temperature of -20 or $-25°C$ and an annealing time of 2 h or longer are suggested if the fill depth is 1 cm or more. Annealing conditions can be studied using either frozen solution X-ray diffraction or DSC procedures to evaluate the development of crystallinity (27; Tchessalov et al., unpublished). Annealing often has effects beyond crystallization of solutes.

Annealing above the glass transition temperature of T_g' causes growth of ice crystals, which decreases the product resistance to flow of water vapor and results in shorter primary drying time (28,29). Also, the product specific surface area is reduced, which decreases the water desorption rate in secondary drying and may lead to increased residual moisture content in the final product or demand longer secondary drying (30). Suggested freezing process are summarized below.

Normal Freezing Process for Amorphous Products

1. Load vials onto the shelf and allow to come to 5°C; hold for 15 to 30 min.

2. Cool to $-5°C$ without ice formation and hold for 15 to 30 min (this normally results in improved homogeneity of crystallization, both intra- and inter-vial).

3. Decrease the shelf temperature to a final shelf temperature of $-40°C$ (all solutes in solid state) at about 1°C/min.

4. Hold for 1 h if fill depth is less or equal to 1 cm or 2 h if the fill depth is greater than 1 cm.

Thermal Cycle To Achieve Solute Crystallization (Crystalline Drug or Bulking Agent)

1. Step 1 to 3 as above.

2. Bring product temperature to 10 to 20°C above T_g' but well below the onset of eutectic melt and hold for several hours.

3. Steps 3 and 4 as above freezing process for amorphous products.

Primary Drying

The next step of freeze drying process design is to optimize the product temperature (T_p). The product temperature, which depends on the properties of formulations, shelf temperature and chamber pressure of the freeze dryer, and container system, cannot be directly controlled during primary drying. Therefore, it is difficult to optimize the freeze-drying process for a given pharmaceutical formulation even when its T_c and T_g' are known. Because primary drying normally consumes the largest fraction of the freeze-drying cycle time, optimization of this portion of the process has significant economic impact (2). Even with highly skilled development scientists, optimization of primary drying can require a number of time-consuming experimental studies. Consequently, many formulations are freeze dried using conditions that are far from optimum. Nonoptimized freeze-drying processes may enormously increase the process time and may compromise product quality and/or produce regulatory concerns. The philosophy of primary drying is to choose the optimum target product temperature (T_p), bring the product to the target product temperature quickly, and hold the product temperature roughly constant at the target temperature throughout all of primary drying.

Target Product Temperature

The product temperature should always be several degrees below T_c in order to obtain a dry product with acceptable appearance. The temperature difference between T_p and T_c is called the temperature safety margin. It is well-known that high product temperature yields a fast process, with each 1°C increase in product temperature decreasing primary time by about 13% (1). Therefore, an optimized freeze-drying process runs with the product temperature as high as possible (2). In other words, the target product temperature should be as close as possible to T_c. However, the risk of collapse is high if product temperature is too close to T_c. Consequently, the optimum target product is a compromise between safety and freeze-drying time. We suggest that a small safety margin (2°C) be used if freeze-drying time is long (e.g., more than 2 days), a large safety margin (5°C) be used if freeze-drying time is short (<10 h), and a safety margin of 3°C be used if primary drying time is somewhere between 2 days and 10 h. In general, T_p should not be higher than $-15°C$ or the heat and

mass transfer capabilities of the freeze dryer may be over-loaded. Overloading of the freeze dryer typically causes loss of chamber pressure control and product temperatures in excess of the target. Overloading of the freeze dryer may occur for both laboratory and manufacturing freeze dryers. Mass flow overloading is common for some laboratory freeze dryers where the connection pathway between the freeze-drying chamber and condenser is too small to handle the high rate of mass transfer. Overloading of manufacturing freeze dryers normally arises from limited heat flow caused by an undersized refrigeration system (Rambhatla and Pikal, unpublished).

Target Product Temperature for Protein Formulations with Low T_g'

Current dogma requires that protein formulations be freeze dried at a temperature lower than both T_c and T_g' (2). It is also assumed that protein drugs are unstable above T_g' but stable below T_g'. This assumption is based on the concept that the rate of unfolding is fast above T_g' but very slow in the "solid state" below T_g'. However, data directly supporting this concept are not available. Freeze drying below T_g' often demands a very low target product temperature and therefore a very long process, particularly when the protein formulation has a very low T_g' due to a formulation containing both sucrose (or trehalose) and salts.

Protein drug formulations usually contain stabilizers such as sucrose or trehalose, which have high viscosity after freeze concentration. It has been reported that the folding kinetics of GCN4-P2', a simple α-helical coil derived from the leucine zipper region of bZIP transcriptional activator GCN4, was correlated to system viscosity in relatively low viscosity solutions (31). Assuming unfolding involves some viscous flow of surrounding solvent and assuming validity of of the Stokes–Einstein relationship [i.e., the reaction rate is inversely proportional to system viscosity (12,32)], the high viscosity of a polyol-rich system may decrease the protein denaturation rate to a degree that the unfolding cannot occur in the time frame of freeze drying, even if freeze dried at a temperature higher than T_g'. However, one still needs to avoid collapse. Collapse may be avoided by using an excess of bulking agent (i.e., mannitol or glycine). The formulation with both stabilizer and crystalline bulking agent has a collapse temperature close to the eutectic temperature of the bulking agent added (eutectic temperature for glycine, or mannitol ≈ –3°C), which is typically much higher than T_g' of most stabilizers (T_g' for sucrose, trehalose ≈ –34°C) (Chang et al., unpublished). Several recent reports show that at least some protein formulations can be freeze dried at temperatures higher than T_g' without damaging stability (29,33), so there is some empirical evidence to support the above theoretical concepts.

Thus, theoretical considerations and some data suggest that provided there exists a high degree of coupling between protein unfolding kinetics and viscosity, the unfolding rate should be negligibly small in saccharide systems even when the system is thermodynamically unstable and the temperature is above T_g'. The correlation between the unfolding half life and viscosity is expected to be of the form (34):

$$t_{1/2} = A \cdot \eta^\alpha$$

where A is a constant of proportionality, η is the system viscosity, and α is the coupling constant. The following (Table I)

Table I. Calculated Protein Unfolding Half-Life ($t_{1/2}$) in Sucrose Freeze Concentrate

% Sucrose (w/w)	T (°C)	$t_{1/2}$ (h), $\alpha = 1$	$t_{1/2}$ (h), $\alpha = 0.5$
65	–15	1.24×10^3	3.68×10^2
70	–19	1.05×10^4	1.12×10^3
75	–24	2.97×10^5	5.97×10^3
80	–30	2.10×10^8	1.59×10^5

At a sucrose concentration of 80% w/w, the systems are at the maximum freeze concentrate composition and the sucrose concentrations are from the equilibrium liquidus line. The viscosity data are from the literature (46).

was calculated for freeze concentrates of a hypothetical protein and sucrose at different temperatures. The calculated results suggest that there is not enough time for the protein to unfold before it is dried even though it is freeze dried at –15°C, which is more than 20°C higher than its T_g'. Of course, these calculations assume strong coupling ($\alpha \geq 0.5$) between unfolding kinetics and viscosity. Recent studies in this laboratory indicate strong coupling, at least for the proteins studied. We find the α values for phosphoglycerate kinase and β-lactoglobulin are 1.94 and 0.68, respectively (Tang and Pikal, unpublished)

Therefore, it is worthwhile to freeze-dry low T_g' protein formulations at a target product temperature higher than its T_g' (using a crystalline bulking agent to avoid macroscopic collapse) and compare its stability with the results of samples freeze dried below T_g'. One may find that instability is not an issue.

Chamber Pressure

Primary drying is carried out at low pressure to improve the rate of ice sublimation. The chamber pressure (P_c) impacts both heat and mass transfer and is an important parameter for freeze-drying process design. P_c should be well below the ice vapor pressure at the target product temperature to allow a high sublimation rate. The sublimation rate is the mass of ice sublimed (g) per unit time (hour), which can be represented by Eq. (1).

$$\frac{dm}{dt} = \frac{P_{ice} - P_c}{R_p + R_s} \tag{1}$$

where, dm/dt is ice sublimation rate (g/hour per vial), P_{ice} is the equilibrium vapor pressure of ice at the sublimation interface temperature (Torr), and R_p and R_s are the dry layer and stopper resistance, respectively, to water vapor transport from the sublimation interface (Torr·h/g) (28). [Throughout this work, we use units consistent with those used on laboratory and commercial freeze-drying equipment in the USA. Thus, the pressure unit used is Torr (or mTorr), rather than the SI unit of Pascals (Pa). The reader is reminded that 0.1 Torr is 100 mTorr and 13.3 Pa.] With this choice of units, the magnitude of the area normalized resistance represents (roughly) the time, in hours, required to freeze dry a 1-cm-thick frozen product at a temperature of –20°C, provided the resistance were to remain constant. The area normalized product resistance ($\hat{R}_p$) and resistance (R_p) are related by,

$\hat{R}_p = R_p \cdot A_p$, where A_p is the cross-sectional area of the product.

The sublimation rate is proportional to pressure difference between the vapor pressure of ice and the partial pressure of water in the chamber (P_i), this difference being the driving force for ice sublimation. P_i is essentially the same as chamber pressure during primary drying. At given product temperature (i.e., given ice vapor pressure), the smallest chamber pressure gives the highest ice sublimation rate. However, very low chamber pressure may cause problems, such as contamination of product with volatile stopper components or pump oil (35), and also produce larger heterogeneity in heat transfer, thereby giving larger product temperature heterogeneity between vials (36). In most applications of practical interest, the chamber pressure varies from 50 to 200 mTorr. It is difficult to maintain consistently chamber pressure much below 50 mTorrr, and there is little reason to use pressures much higher than 200 mTorr. It has been reported that moderate chamber pressure (100–150 mTorr) gives optimal homogeneity of heat transfer in a set of vials (36). Therefore, the optimum chamber pressure is a compromise between high sublimation rate and homogenous heat transfer. Equation (2) may be used to chose the "optimal" chamber pressure at known target product temperature (T_p).

$$P_c = 0.29 \cdot 10^{(0.019 \cdot T_p)} \qquad (2)$$

where P_c is chamber pressure (Torr) and T_p is product temperature (°C).

Shelf Temperature Required To Achieve Target Product Temperature

Often, the most time-consuming part of freeze-drying process design is the determination of the shelf temperature: time profile that efficiently achieves the target product temperature during primary drying. The product temperature during primary drying is typically 5 to 40°C lower than shelf temperature and changes with chamber pressure, shelf temperature, heat transfer coefficient of the container (typically, vials), thermal history of the formulation, and even with the freeze dryer. At constant chamber pressure and shelf temperature, the product temperature changes as drying progresses, often increasing 1 to 3°C from beginning to the end of primary drying (36). Product temperature can be measured by several methods, including thermocouples, or RTD temperature sensors in selected product vials, and by pressure rise measurement (manometric temperature measurement) (37). Manometric temperature measurement (MTM) is a procedure to measure the product temperature at the sublimation interface during primary drying by quickly isolating the freeze-drying chamber from the condenser for a short time and subsequent analysis of the pressure rise during this period by fitting a theoretical relationship (MTM equation) to the pressure rise data (37–39). The thermocouple or RTD method measures the product temperature at the bottom of vials whereas the MTM method measures the product temperature at the ice sublimation interface, which is usually 0.5 to 2°C lower than at the vial bottom. The MTM method has the advantage of minimal human intervention during set-up of the freeze-drying process (i.e., no need for an operator to place manually temperature sensors in the vials). Also, MTM analysis yields accurate product resistance, which can be used

to characterize the heat and mass transfer in real time during primary drying (38). This information may be combined with heat and mass transfer equations to allow the calculation of the vial heat transfer coefficient and, thus, to develop an optimum freeze-drying cycle during the very first freeze-drying experiment (39).

The product temperature is usually higher at the front and side or back and colder in the interior, a result of additional radiation heat transfer from the door and chamber walls to the edge vials (36). This product temperature heterogeneity can be minimized by use of thermal or radiation shields, such as empty vials around the sample vials and aluminum foil on the inside of the chamber door (39). For a given formulation with a given target product temperature, the shelf temperature can be estimated by using coupled steady-state heat and mass transfer theory. The heat transfer rate to vials may be described by Eq. (3) (36).

$$\frac{dQ}{dt} = 3600 \cdot A_v \cdot K_v \cdot (T_s - T_b) \qquad (3)$$

where Q is energy received by each vial from the shelf (cal/vial), dQ/dt is heat transfer rate (cal/hour per vial), A_v is the outer area of vial bottom (cm^2), K_v is heat transfer coefficient (cal/s·cm^2·K), T_b is product temperature (°C) at the bottom of the vial, T_s is shelf temperature (°C), and 3600 arises from converstion of the heat flow units from cal/s per vial into cal/h per vial. The shelf temperature required to obtain a given product temperature may be calculated from a combination of Eqs. (3) and (4) in the form (36,39),

$$\frac{dQ}{dt} = \Delta H_s \cdot \frac{dm}{dt} \qquad (4)$$

$$T_s = T_p + \frac{1}{A_v} \cdot \frac{dQ}{dt} \cdot \left(\frac{1}{K_v} + \frac{l_{ice}}{k_I} \right) \qquad (5)$$

where ΔH_s is heat of ice sublimation (cal/g), l_{ice} is ice thickness (cm), and k_I is the thermal conductivity of ice (cal·K^{-1}·cm^{-1}·s^{-1}). The term in l_{ice} accounts for the temperature difference across the frozen layer. The heat transfer rate or heat flow in Eq. (5) is dependent on target product temperature at the sublimation interface (T_p) and product resistance (R_p). The product resistance is formulation and concentration dependent with higher solute concentration yielding higher product resistance. The heat transfer coefficient of vials (K_v) mainly depends on the type of the vial and chamber pressure, and can be represented by Eq. (6) (36),

$$K_v = KC + \frac{3.32 \cdot 10^{-3} \cdot P}{1 + KD \cdot P} \qquad (6)$$

where the first term, KC, is the sum of the contact and the radiative heat transfer parameters (cal/s·cm^2·K), and the second term expresses the heat transfer from the shelf to vial bottom by gas conductivity, in which P is the gas pressure (Torr) and KD is a parameter that is related to the average distance between the shelf and the vial bottom (40). The tubing vials have greater heat transfer by contact conductivity (i.e., greater KC values) and greater gas conductivity (i.e., smaller KD values) than molded vials. Representative data for KC and KD are given in Table II. As a good approximation, high solute concentration (>10%) corresponds to high area normalized product resistance (5 Torr·cm^2·h/g), and low

Table II. Vial Heat Transfer Parameters*

Type	A_v (cm²)	A_p (cm²)	$10^4\ KC$	KD
10 cc tubing	4.71	3.80	2.64	3.64
20 cc tubing	6.83	5.72	2.03	3.97
30 cc molded	8.31	6.07	1.82	5.18
100 cc molded	17.2	14.3	1.52	6.97

A_v is the outside cross-sectional area of vials, and A_p is the inside cross-sectional area of vials. KC has the same unit as K_v (cal/sec·cm²·K) and KD has the unit Torr⁻¹.
* Data from Ref. 40.

solute concentration (<1%) corresponds to low area normalized resistance (1 Torr·cm²·h/g) (28). Thus, resistance may roughly be estimated from solute concentrations. For a given target product temperature (T_p) (therefore given P_{ice}), the optimum chamber pressure (P_c) is calculated by Eq. (2). Next, the mass transfer rate (dm/dt) is calculated by Eq. (1), the heat transfer rate (dQ/dt) is calculated by Eq. (4), the vial heat transfer coefficient is calculated by Eq. (6) (given P_c and type of vials), and finally the shelf temperature needed to achieve the target product temperature can then be evaluated from Eq. (5). The calculated shelf temperatures required to achieve the target product temperatures are plotted in Fig. 1. These data assume tubing vials are used. This chart, although not highly accurate, can be used as a guideline for initial selection of shelf temperature using only limited formulation data. Once the process starts, the shelf temperature needs to be readjusted if the product temperature deviates from the target temperature. Because non-steady-state effects persist for about one-half hour after a shelf temperature change, the shelf temperature should not be increased until after the shelf temperature is stable for at least half an hour. As a rough rule,

product temperature changes by 1–2°C for each 5°C change of shelf temperature. The shelf temperature is kept constant until product temperature exceeds the target product temperature by a predetermined tolerance (1 or 2°C). The adjustment of shelf temperature is not recommended after primary drying is more than two-thirds over because not all vials finish primary drying at the same time, and near the end of the process, an increase in product temperature in a thermocouple vial might simply indicate the end of primary drying in that vial. Moreover, once primary drying is about two-thirds finished, additional shelf temperature adjustments are not necessary.

Overloading of Freeze Dryer

Care must be taken not to overload the heat and mass transfer capabilities of the freeze dryer. The arrows in Fig. 1 show the estimated shelf temperature above which the mass flow exceeds 1 kg/h/m², which is roughly the maximum load for a typical manufacturing freeze dryer that can be maintained without risk of overload. Figure 1 is highly approximate. The exact overload conditions of a specific freeze dryer can only be obtained from suitable operational qualification (OQ) data. Figure 2, which is calculated using the same strategy as for Fig. 1, shows the estimated mass flow as a function of calculated shelf temperatures at different product resistances. This figure can be used as a guide if the freeze-dryer overload conditions are known. The real-time heat flow during primary drying can easily be calculated by Eq. (1) if accurate product resistance values from manometric temperature measurement are available (38) or by the heat transfer equation, Eq. (3), if the vial heat transfer coefficient (K_v) and product temperature are known. The vial heat transfer coefficient can be calculated using Eq. (6) for a given vial type (Table II) where the "optimum" chamber pressure (P_c) is calculated from Eq. (2) (36).
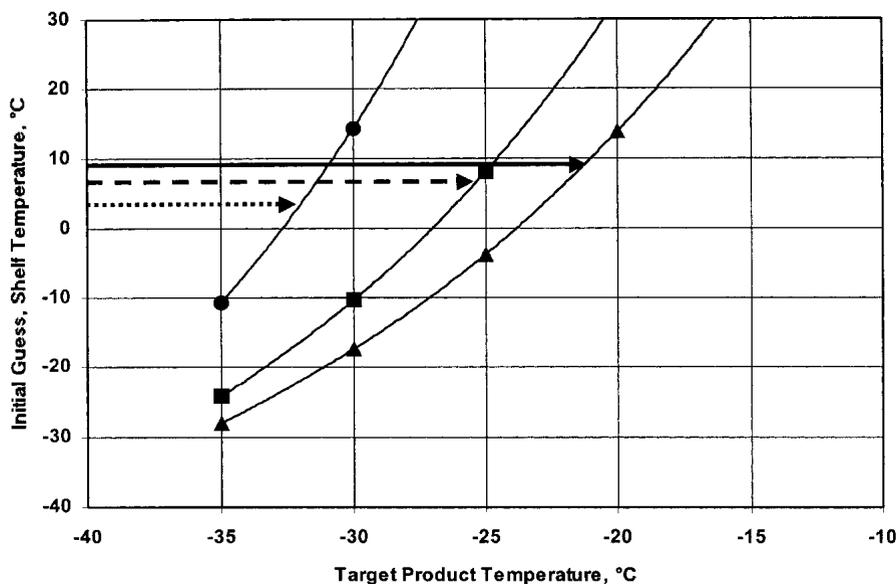

Fig. 1. Initial shelf temperature estimation for given target product temperatures at different solute concentrations (i.e., different dry layer resistance in primary drying): circles = low dry layer resistance (solids ≤1%), squares = medium dry layer resistance (10% > solids >1%), triangles = high dry layer resistance (solids ≥10%). Calculations used Eqs. (1)–(5) as described in the text. The arrows specify the maximum shelf temperatures above which mass and heat transfer overload of a "typical" manufacturing dryer is likely.
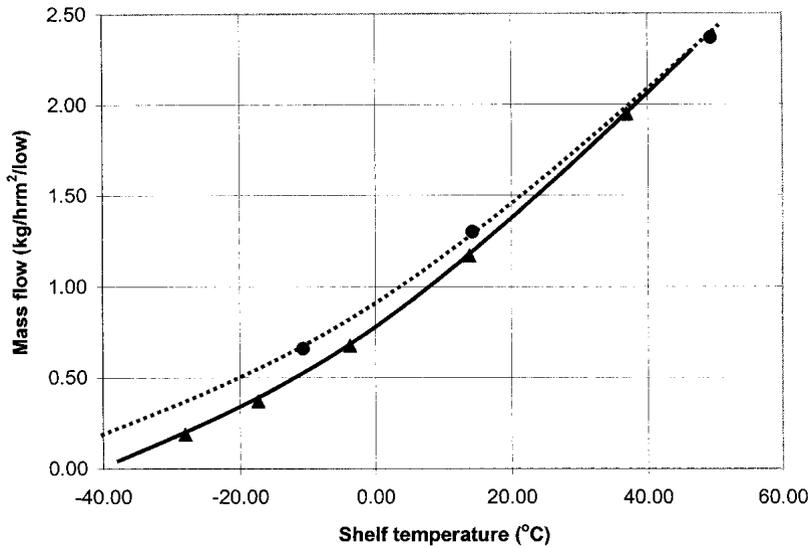
Fig. 2. Estimated mass flow in primary drying as a function of shelf temperature at different product resistance: circles = low dry layer resistance (solids $\leq 1\%$), triangles = high dry layer resistance (solids $\geq 10\%$). Calculations performed using Eqs. (1)–(4) and a procedure similar to that used to generate Fig. 1.

If at any time during primary drying the calculated heat or mass flow is close to the overload value, the shelf temperature should be adjusted down. Thus, the condenser overloading problem can be avoided if real-time heat or mass flow data are available (i.e., from MTM data or any other source), and the overload sublimation rate is known.

Primary Drying End Point

The primary drying time is directly related to the ice sublimation rate and is determined by numerous factors, including chamber pressure, shelf temperature, heat transfer coefficient of vials, fill volume, and product resistance (40). The primary drying time can roughly be estimated from the calculation of heat and mass flow via Eqs. (3) and (4). Primary drying time estimates for tubing vials are plotted in Fig. 3 as a function of $T_s - T_b$. These estimates are crude approximations, but can be used as an initial guess of the primary drying time with little input data required.

The end point of primary drying can be detected by several different methods. At the end of primary drying, there is no ice present in vials (i.e., no ice sublimation and no heat removal by sublimation); thus, the product temperature increases to the shelf temperature, and the vapor composition in the freeze-drying chamber changes from essentially all water vapor during primary drying to mostly air or nitrogen (36). Thus, product temperature data indicate the end point of primary drying when the product temperature approaches the shelf temperature. Normally, the product temperature as a function of time shows a steep increase in temperature at the end of primary drying, followed by a plateau. Frequently, the vials containing thermocouples are not representative of the batch as a whole because of the bias in freezing behavior. Thermocouple vials usually have less supercooling, larger ice crystals, and faster ice sublimation, and, therefore, shorter primary drying time (41). Drying differences between thermocouple vials and the rest of the batch could be a serious problem, especially for manufacturing where the bias in freez-

ing behavior is more pronounced. Also, in manufacturing, thermocouples are usually placed in front-row vials for maximum sterility assurance. Front-row vials finish primary drying far earlier than the interior vials due to atypical radiation heat transfer effects (36). Thus, thermocouple vials are not representative both because of heat transfer effects and because of freezing-bias-induced mass transfer effects. Among the other methods that can be used, those methods that sense the transition in gas composition in the chamber from water vapor to nitrogen are perhaps easiest to use. Dew point sensors, which can detect the vapor composition change or the relative humidity in the freeze-drying chamber, shows a sharp dew point
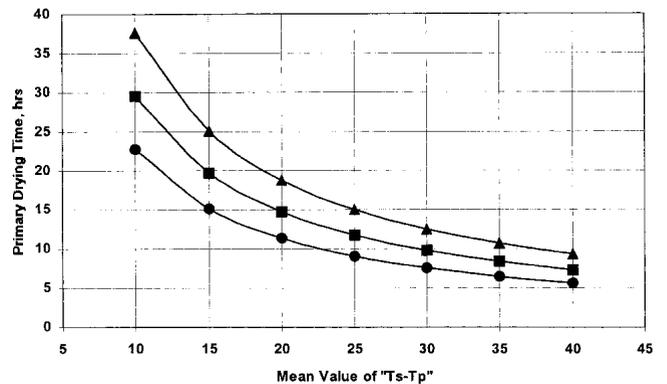

Fig. 3. Estimated primary drying time as a function of temperature driving force for different solute concentrations: circles = low dry layer resistance (solids $\leq 1\%$), squares = medium dry layer resistance (10% > solids >1%), triangles = high dry layer resistance (solids $\geq 10\%$). Calculations were performed using Eqs. (3) and (4), assuming heat transfer coefficients and geometry typical for tubing vials and assuming a 1-cm-thick frozen cake. In this approximate calculation, drying time is proportional to frozen cake thickness and inversely proportional to $T_s - T_p$. To use Fig. 3, the shelf temperature, T_s, is estimated using Fig. 1 as instructed in the text, and for the rough estimate provided by Fig. 3, the target product temperature, T_p, may be substituted for T_b.

decrease at the end of primary drying due to the vapor compositions in the chamber changing from almost 100% water vapor to essentially 100% nitrogen. Comparative pressure measurement also works well. The thermal conductivity pressure gauge (Pirani pressure gauge) is calibrated against air and shows higher vapor pressure during primary drying because the thermal conductivity of water vapor is about 1.5 times that of air or nitrogen. At the end of primary drying, the pressure difference between the thermal conductivity pressure gauge (Pirani gauge) and capacitance pressure gauge (MKS Baratron gauge, which measures actual pressure) decreases and approaches zero (9,41). A variation of the classical pressure rise test, called the MTM method (39), is another very sensitive and promising method to indicate the end point of primary drying. The vapor pressure of ice determined by a fit of pressure rise data to the MTM equations approaches the chamber pressure when no ice remains (39). The MTM method differs from the classical pressure rise test in that the MTM procedure removes the impact of water desorption from the freeze concentrate, and does not require "calibration" for each application to determine how much pressure rise is needed for a "not dry" decision. Experiments have shown that the MTM method can detect the pressure of only three vials with ice in a 50-liter laboratory freeze dryer (39).

When thermocouple vials are used to indicate the end point of primary drying, an additional "soak time" or safety margin for primary drying time of at least 10% of the primary drying time of the thermocouple vials is helpful to make sure all ice has been removed. The soak time is the period of extended primary drying after the end point of primary drying as determined by thermocouple response. This soak time requirement is due primarily to the bias in primary drying behavior between thermocouple vials and the rest of the batch. Here we suppose the thermocouple vials are placed in the center of the array. The drying bias is larger if the thermocouples are placed in the front row, as is typical, and a longer "soak time" is necessary, at least 20% of the primary drying time for thermocouple vials. A soak time is not needed with the other methods of end point detection.

The primary drying time is usually longer for manufacturing freeze drying due to greater supercooling in the sterile and "particle free" environment in manufacturing as well as less radiation heat transfer from the door and walls. If laboratory-developed processes that are based on a fixed time for primary drying are used without modification, there is a high risk of melt-back or collapse problems. The melt-back or collapse problem results from shelf temperature elevation before the end of primary drying, and the resulting increase in product temperature melts or at least collapses the product at the vial bottom. We suggest extending the laboratory drying time by at least 20% to scale-up processes that do not use annealing and thus have the potential for large freezing bias.

Secondary Drying

The last stage of freeze drying is termed "secondary drying." In this stage, water that did not freeze is removed by desorption from the solute phase. Immediately after primary drying, an amorphous product still contains a fair amount of residual water (5–20% on a dried solids basis, depending on the formulation). The objective of secondary drying is to reduce the residual moisture content to a level optimal for sta-

bility, which is usually less than 1%. The shelf temperature in secondary drying is much higher than that used for primary drying so that desorption of water may occur at a practical rate.

Heating Rate and Chamber Pressure

The shelf temperature should be increased slowly for secondary drying because a fast temperature ramp might cause collapse of amorphous products. Because of the fairly high residual moisture content in the amorphous product early in secondary drying and, thus, low glass transition temperature, the potential for collapse is greatest early in secondary drying. A ramp rate of 0.1 or 0.15°C/min for amorphous products is generally a safe and appropriate procedure, but this slow ramp rate need not be maintained once the product temperature reaches ambient temperature. Crystalline products do not have any potential for collapse during secondary drying, and a higher ramp rate is suggested for such products (0.3 or 0.4°C/min). The water desorption rate does not depend on chamber pressure, at least if the chamber pressure is less than about 200 mTorr, but is very sensitive to product temperature (30). Because the chamber pressure in primary drying is also appropriate for secondary drying, it is not necessary to change chamber pressure for secondary drying.

The Shelf Temperature and Secondary Drying Time

The products should be kept at "high" temperature for a period sufficient to allow the desired water desorption. Usually, it is better to run a high shelf temperature for a short time than a low temperature for a long period (30). The reason is that the water desorption rate decreases dramatically with time at a given temperature and times longer than 3–6 h at a given temperature do little to further reduce the moisture content. Amorphous products are more difficult to dry than crystalline products; thus, higher temperatures and longer times are needed to remove the absorbed water. The secondary drying conditions also depend on the solute concentration. At higher solute concentration (i.e., >10% solids in solution), the dry product has smaller specific area, and it is more difficult to remove the absorbed water; thus longer times and/or higher temperatures are needed to finish secondary drying. Normally, drying times of 3–6 h at the terminal temperature is optimal with the terminal temperature varying accordingly to the formulation, but is normally in the range 40 to 50°C, even for proteins. Note that protein denaturation in the relatively dry solid state is not an issue below about 100°C (Pikal et al, unpublished). [We have observed protein denaturation in the relatively dry (<15% water) solid state to be well over 100°C for human growth hormone (42; Pikal et al., unpublished) as well as with several other proteins, and when the formulation is very dry, the denaturation temperature may be over 150°C. Hageman (43) makes much the same observation for bovine growth hormone.] The optimum secondary drying time can be determined by real-time residual moisture measurement during secondary drying by extracting samples from the freeze dryer without interrupting the freeze drying (i.e., use of a "sample thief") and measuring their moisture content using Karl Fischer titration (KF), thermal gravimetric analysis (TGA), or near IR spectroscopy (44).

The real-time (*in situ*) moisture content can also be measured by a modified MTM method provided the actual moisture content at the end of primary drying is measured (39). Near IR also has potential for "in-process" measurement of water content if the working standard curve is well calibrated (45).

It is well-known that high water content normally decreases the storage stability of drugs. Therefore, the product is usually freeze dried to very low residual moisture content (about 0.5%). However, low moisture content in freeze-dried products does not guarantee best storage stability, at least for proteins and more complex biological products. On rare occasions, there is an intermediate moisture content for which the product has optimal storage stability (42,43). If the target moisture content is an intermediate level, design of secondary drying is more difficult. Usually, a combination of long drying times (6 h) and low shelf temperature (about 0°C) are best, but the exact conditions must be determined by trial and error.

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