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Evaluation of Manometric Temperature Measurement, a Process Analytical Technology Tool for Freeze-Drying: Part I, Product Temperature Measurement

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ABSTRACT

This study examines the factors that may cause systematic errors in the manometric temperature measurement (MTM) procedure used to evaluate product temperature during primary drying. MTM was conducted during primary drying using different vial loads, and the MTM product temperatures were compared with temperatures directly measured by thermocouples. To clarify the impact of freeze-drying load on MTM product temperature, simulation of the MTM vapor pressure rise was performed, and the results were compared with the experimental results. The effect of product temperature heterogeneity in MTM product temperature determination was investigated by comparing the MTM product temperatures with directly measured thermocouple product temperatures in systems differing in temperature heterogeneity. Both the simulated and experimental results showed that at least 50 vials (5 mL) were needed to give sufficiently rapid pressure rise during the MTM data collection period (25 seconds) in the freeze dryer, to allow accurate determination of the product temperature. The product temperature is location dependent, with higher temperature for vials on the edge of the array and lower temperature for the vials in the center of the array. The product temperature heterogeneity is also dependent upon the freeze-drying conditions. In product temperature heterogeneous systems, MTM measures a temperature close to the coldest product temperature, even if only a small fraction of the samples have the coldest product temperature. The MTM method is valid even at very low product temperature (−45°C).

KEYWORDS: freeze-drying/lyophilization, manometric temperature measurement, process analytical technology for freeze drying, product temperature heterogeneity.

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INTRODUCTION

Freeze-drying is widely used with pharmaceuticals to improve the long-term storage stability of labile drugs, especially protein drugs.[1,2] During both freeze-drying process development and production, product temperature control is extremely important. In general, product temperature in primary drying should be below the collapse temperature (T_c) or the glass transition temperature of the frozen solution (T_g'), otherwise gross collapse will occur.[3,4] Accurate measurement of product temperature during primary drying is a critical process analytical need. Primary drying, or the ice sublimation stage of freeze-drying, is executed under reduced chamber pressure to facilitate 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 subsequent crystallization onto the cold coils/plates (< −50°C) in the condenser.

The product temperature results from a balance between the heat input from the shelves and self-cooling from ice sublimation, and thus depends on the properties of formulations, the shelf temperature, chamber pressure, and the container used. Product temperature cannot be controlled directly during primary drying but is controlled by change of shelf temperature and chamber pressure.[3] The conventional method of measuring product temperature during freeze-drying is to place temperature sensors, such as thermocouples or electric resistance temperature detectors (RTDs) directly inside the sample vials.[5] However, placement of sensors in vials might compromise sterility because the sensor must be placed inside the vial by hand. Temperature sensors inside sample vials also induce ice nucleation and, therefore, cause bias in both freezing and drying behavior relative to the vials not containing temperature sensors.[6] Moreover, the temperature sensors placed inside the vials (usually placed at the bottom of the vials) do not determine the product temperature at the sublimation interface, which is more critically related to product collapse in primary drying. As an alternative to placement of sensors in vials, manometric temperature measurement (MTM) has been suggested for monitoring the product temperature during primary drying.[7]

MTM is a procedure by which product temperature at the sublimation interface may be measured during primary drying without placing any device in the vial. With MTM, the valve between chamber and condenser is quickly closed, thereby isolating the freeze-drying chamber from the condenser for a short time. The MTM method records the pressure versus time data and analyzes the data to calculate the temperature at the sublimation interface.[7] Unlike conventional methods, which can take temperature readings in different vials at different locations, the MTM method yields only one temperature value. However, inter-vial temperature heterogeneity, which is usually location specific in primary drying, is a common phenomenon during primary drying.[3] While it seems obvious that the MTM temperature represents some type of average for the system, it is not known whether this average is weighted in favor of the higher temperature vials, the lower temperature vials, or is a simple numerical average.

It was suggested that the lowest product temperature that can be reliably measured by MTM method is about −35°C.[7] However, data that allowed testing this suggestion were not available. Many pharmaceuticals, especially protein formulations, are freeze-dried below −35°C, and it is not uncommon to freeze-dry a protein formulation at product temperature as low as −40°C.[8] Therefore, a lower temperature limit of −35°C would be a serious limitation to MTM in practical freeze-drying. Additional studies are needed to assess the true lower limit of the MTM procedure. Furthermore, other limitations on use of the method and the effect of instrument and process conditions on systematic errors were not extensively explored in the earlier work.[7]

The time profile of the MTM data, which is a vapor pressure rise as a function of time, consists of 2 phases (ie, a fast rise phase and a plateau phase). The product dry layer resistance, the ice sublimation area, and the chamber volume define the rate of vapor pressure rise in the fast rise phase. A high product dry-layer resistance, large chamber volume, and small ice-sublimation area give a slow vapor pressure rise. One might expect a slow pressure rise to negatively affect MTM data analysis (ie, result in MTM product temperatures that are in error). The problem would be most serious when small numbers of vials are used with a high dry-layer resistance product for freeze-drying in a relatively large freeze-dryer. In this case, if the rate were too slow for the plateau phase to be reached by the end of the measurement period, MTM product temperature data would likely be subject to errors. Indeed, we have observed MTM product temperatures being too low when a small load of moderately high dry-layer resistance material (5% mannitol) was freeze-dried. Of course, the duration of the valve closure could always be prolonged to allow a plateau region to develop. However, since the product temperature is always significantly below the shelf temperature during primary drying, closing the valve between chamber and condenser, which slows down the ice sublimation, will increase the product temperature. The product temperature increase during the MTM data collection period ultimately limits the valve closure time and therefore imposes a limitation on the chamber pressure rise. Therefore, the effects of ice-sublimation area, the dry-layer resistance, and chamber volume on the reliability of the MTM method need to be evaluated to optimize the MTM procedure.

A well-developed and reliable MTM procedure would be a powerful freeze-drying process analytical technology (PAT). Such a procedure would provide a means of monitoring product temperature as well as heat and mass transfer in real time during freeze-drying. Accurate product temperature measurement is a critical PAT, given the sensitivity of process economics and product quality to product temperature history. We also suggest that measurement of mass and heat transfer coefficients can be extremely useful in formulation design and process optimization. This research is a study of those factors that may limit use of the MTM procedure. The effect of product temperature heterogeneity on accuracy of the measured product temperature is studied, limitations in the measurement of very low product temperature are explored, and the effect of measurement time (ie, valve closure time) on product temperature increase is investigated. Further, the effects of ice-sublimation area (or number of vials), product dry-layer resistance, and freeze-drying chamber volume on accuracy of temperature measurement are defined.

Materials and Methods

Sucrose and glycine were purchased from Sigma (St Louis, MO) and used without further purification. All the reagents were analytical grade. All the vials used for freeze-drying were 5-mL serum tubing vials of 20-mm finish from Fisher Scientific (Pittsburgh, PA), and the stoppers were dual vent lyophilization stoppers (Fisher). The outside and inside cross-section areas of the vials (A_v and A_p) are 3.65 and 2.91 cm^2, respectively.

Pressure Gauge and Thermocouple Calibrations

The pressure gauge (capacitance manometer, MKS Instruments, Andover, MA) with a resolution of ±1 mTorr was calibrated against our "standard," an MKS Baratron, type 690, high-accuracy, absolute capacitance manometer in the range from 0 to 2000 mTorr. The 28-gauge Copper-Constantan (T type) thermocouple temperature gauges (Omega Engineering, Stamford, CT) with a resolution of ±1°C were calibrated at 0°C using ice-water system.

Freeze-drying

Freeze-drying was performed with an FTS Dura-Stop/Dura-Top freeze-drier with the manometric temperature measurement software installed. A 2-mL fill in 5-mL vials was used, with all vials loaded on the middle shelf of the freeze-dryer. Radiation heat shields were used for some experiments including empty (dummy) vials around sample vials to minimize radiation heat transfer from the freeze-dryer chamber wall and the door, and aluminum foil was attached to the inside of the chamber door to minimize radiation from the door and outside. The number of vials used depended upon the experimental design. Note that different numbers of vials represented different ice-sublimation areas.

The freeze-drying cycles for 50 mg/mL glycine were (1) freezing: cool 1°C/min to 5°C, hold for 30 minutes; cool 1°C/min to −25°C, hold for 60 minutes; cool 1°C/min to −40°C, hold for 60 minutes; and (2) primary drying: chamber pressure 80 mTorr; ramp 1°C/min to assigned shelf temperature, hold until primary drying is completed.

The freeze-drying cycles for 50 mg/mL sucrose were (1) freezing: cool 1°C/min to 5°C, hold for 30 minutes; cool 1°C/min to −40°C, hold for 60 minutes; and (2) primary drying: chamber pressure 80 mTorr; ramp 1°C/min to assigned shelf temperature, hold until primary drying is completed.

Manometric Temperature Measurement

MTM measurements were made at 1-hour intervals during primary drying, and pressure data were collected at the rate of 4 points per second during the MTM measurement. Typically, the valve is closed and data are collected for 25 seconds. The MTM equation (Equation 1) was fit to the data, which are chamber pressure as a function of time, by nonlinear regression analysis to obtain the vapor pressure of ice at the sublimation temperature, P_{ice}, and the sum of the normalized product and stopper resistance, $R_p + R_s$, using a commercial software package (Microcal Corp., Northampton, MA).

The MTM equation describing chamber pressure rise, P(t), as a function of time, t, during the procedure may be written as Equation 1).[7]

$$P(t) = P_{ice} - (P_{ice} - P_0)\cdot exp\left[-\left(\frac{3.461\cdot N\cdot A\cdot T_s}{V\cdot(R_p + R_s)}\right)\cdot t\right]_{Term\ 1} + 0.0465\cdot P_{ice}\cdot\Delta T \cdot\left[1 - 0.811\cdot exp\left(-\frac{0.114}{L'}\cdot t\right)\right]_{Term\ 2} + \underbrace{X\cdot t}_{Term\ 3} \quad (1)$$

where P_{ice} is vapor pressure of ice at the sublimation interface (parameter to be determined, or "fit"); P_0, is the chamber pressure (set); N is the total number of sample vials (known); A is the inner cross-section area of vials (known); T_s is shelf temperature (set); V is the freeze-drying chamber volume (known); $R_p + R_s$ is the total area of normalized product and stopper resistance (parameter to be determined, or "fit"); ΔT is the temperature difference across the frozen layer (here, we use a fixed value of 1K); L' is the ice thickness (known or calculated from previous data); and X is a constant (parameter to be determined, or "fit"). While Equation 1 is a theoretical result based upon phenomenological heat and mass transfer concepts, as with all theories, it is an approximation and therefore subject to some limitations. Indeed, it is the purpose of this study to experimentally explore these limitations.

In the MTM equation, the "N · A" term is the total area of sublimation interface, and the "(N·A)/ V" term is the ice area to chamber volume ratio or "volume normalized area." In Equation 2, a collection of terms, Q, are identified as important in defining the rate of pressure increase as

$$Q \equiv \left(\frac{3.461\cdot N\cdot A\cdot Ts}{V\cdot(R_p + R_s)}\right) \quad (2)$$

The MTM pressure rise is attributed to 3 parts: (1) the pressure rise controlled by dry-layer resistance and the ice temperature at the sublimation surface described by "term 1" in the MTM equation; (2) the pressure rise caused by the temperature increase at the sublimation surface arising from the dissipation of the temperature gradient across the frozen layer, described by "term 2" in the MTM equation; and (3) the pressure rise due to the increase in ice temperature by heat transfer from the shelf during the MTM procedure (ie, heat continues to flow from shelf to product), described by the linear term "term 3" in the MTM equation, X · t. This linear term also includes the effect of an air leak, but air leaks are normally negligible.

Fitting Equation 1 to the pressure rise data yields the vapor pressure of ice at the sublimation interface (P_{ice}) and the total resistance of product and stoppers ($R_p + R_s$). The MTM product temperature is calculated from the vapor pressure of ice (P_{ice}) by Equation 3.

$$T(MTM) = \frac{-6144.96}{ln(P_{ice}) - 24.01849} \quad (3)$$

where, T(MTM) is the MTM product temperature (K), and P_{ice} is MTM "fitted" vapor pressure of ice (Torr).[4] As an example of a typical good fit of the MTM equation to the pressure rise data, Figure 1 shows data obtained during a 50-mg/mL glycine freeze-drying experiment.

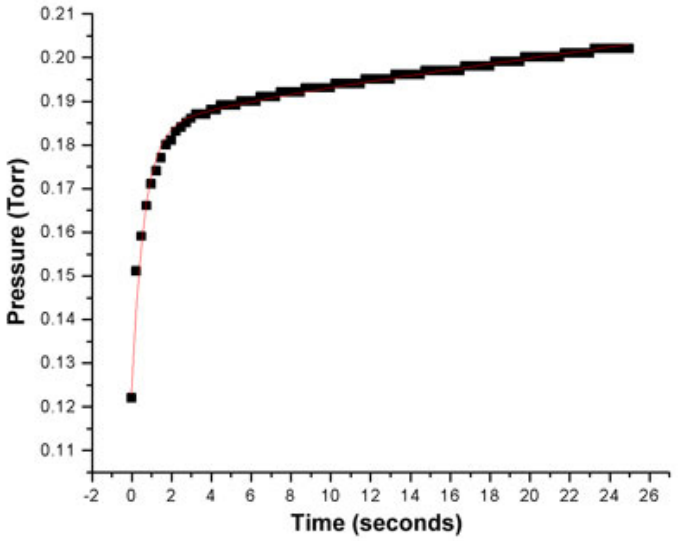

Figure 1. Illustration of the fit of the MTM equation to pressure rise data: freeze-drying of 5% glycine, 2 mL fill in 150 (5 mL) tubing vials, shelf temperature −20°C, chamber pressure 80 mTorr. Symbols are raw data and the line is fitted P(t). The parameters in MTM equation are P_0 = 0.089 Torr; P_{ice} = 0.177 Torr; N = 150; A = 2.91 cm^2; T_s = 253 K; V = 52 L; $R_p + R_s$ = 2.8 Torr·hour·cm^2·/g; L'= 0.55 cm; and X = 0.00058 Torr/s.

Calculation of Product Temperature at the Sublimation Interface From Thermocouple Data

Since thermocouples measure the product temperature at the vial bottom, the temperature at the ice-sublimation interface, T, can be calculated by Equation 4 assuming as a first approximation that all the heat consumed by ice sublimation is provided by the shelf (on which the vial is placed).[9]

$$T = T_b - \frac{(dQ/dt)\cdot L'}{Av\cdot\kappa_I} \qquad (4)$$

where *L'* is ice thickness, which is taken as the fill depth of solution divided by ice density at the very beginning of primary drying, κ_I is the thermal conductivity of ice, and T_b is the product temperature at the vial bottom.

Thermocouple Placement

The thermocouple temperatures were measured in vials at different locations in the vial array, including edge vials (front and side vials) and internal vials. Thermocouples were placed in the middle of the vials touching the bottoms in all cases.

Results and Discussion

Optimal Vapor Pressure Rise for Temperature Measurement: Simulation Results

The values of P_{ice} and resistance ($R_p + R_s$) obtained from the experiment described by Figure 1 were used to generate "simulation" pressure rise curves. This experiment was a typical freeze-drying run with an MTM product temperature of −34.5°C and a temperature at the sublimation interface determined by thermocouples of −35°C. The purpose of the chamber pressure rise simulation (in Figure 2) is to illustrate the components contributing to the chamber pressure versus time profile for MTM, such that the physical meaning of the MTM equation (Equation 1) as well as the limitations of the MTM procedure may be better understood. In general, the pressure rise profile can be divided into 2 distinct parts (ie, an exponential part and a nearly linear plateau region, which was previously described as the "product temperature-dominated part") (Figure 2). The exponential part of the vapor pressure rise represents the part of the curve where term 1 in the MTM equation is changing rapidly, and the product temperature-dominated part represents the part of the curve where term 1 is essentially equal to the vapor pressure of ice, P_{ice}. The duration of the exponential part, which depends upon the Q value, is typically only a few seconds long.

A calculation was performed to determine if existence of the product temperature-dominated part is needed for accurate MTM product temperature measurement. For freeze-drying of 50 mg/mL glycine with a load of 150 vials (5 mL), it takes ~7 seconds to complete 99% of the pressure rise from P_0 to P_{ice} for term 1 in the MTM equation. Therefore, an MTM data collection time of 25 seconds is sufficient for a reliable MTM product temperature fit. However, this might not be true if the data collection period is too short (or the resistance-dominated part is too long) to allow the

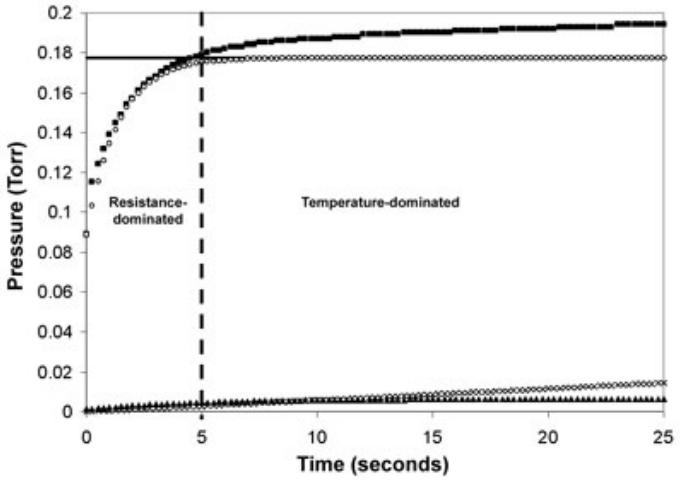

Figure 2. An illustration of the 2 regions of the existence of the pressure rise curve. ■ Experimental pressure rise, P(t); ○ simulated contribution of dry-layer resistance, term 1 in MTM equation; ▲ simulated contribution of ice temperature gradient, term 2 in MTM equation; × simulated contribution of ice temperature increase by shelf and gas leakage, term 3 in MTM equation; horizontal line = P_{ice}.

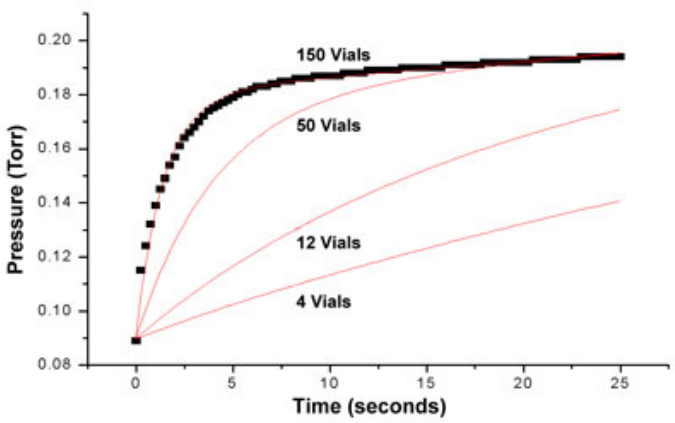

Figure 3. Simulated pressure rise data for variable sample loads. Input parameters are as for Figure 1 except for the number of vials. Symbols are experimental results and smooth curves are simulation results.

temperature-dominated part to appear within the data collection period. For example, if the MTM equation is fitted to only 10 seconds of the MTM pressure-rise data, the MTM temperature is 0.5°C lower than the value determined with the full 25 seconds of data because the product temperature-dominated part is only 3 seconds in duration. Moreover, the MTM temperature is 1.3°C lower than the value determined with 25 seconds data if only 5 seconds of MTM pressure-rise data are used. Here, the product temperature-dominated part of the pressure rise curve never appears. Therefore, the existence of the product temperature-dominated portion of the pressure rise curve is critical for accurate MTM product temperature.

Figure 3 shows the effect of ice-sublimation area (ie, number of vials loaded) on the pressure-rise data. These simulation results were obtained using the same input parameters as used for Figure 2, except for the number of vials. Exponential portions for both 150 and 50 vials were sufficiently short to allow appearance of the product temperature-dominated phase within the 25-second data collection period. However, with 12 and 4 vials, the product temperature-dominated phase never appears (Figure 3).

As can be seen in from Equation 1, the rate of pressure rise is controlled by the value of Q (Equation 2). For a "medium resistance" product (M. Pikal and S. Shah, unpublished observations, 1995),[10] where $R_p + R_s \approx 3$ Torr·hour·cm^2/g, the Q values for 150, 50, 12, and 4 vials are 0.68, 0.23, 0.05, 0.02 g/L·hour·Torr, respectively, with exponential phases of 7, 20, 85, and 255 seconds, respectively. Of course, the Q values will vary with product dry-layer resistance, $R_p + R_s$. Clearly, longer MTM data collection times are required to develop the temperature-dominated phase when the Q value is small (<0.2). However, MTM data collection times much in excess of 30 seconds are unacceptable since the longer time will allow considerable product temperature increase, which might cause product collapse and/or adversely affect product stability. The product temperature increases during the MTM valve closure period are mainly due to heat transfer from shelf to product. The increase in vapor pressure of ice arising from ice temperature increases, which is described by term 3 in the MTM equation, may be obtained from the MTM fit results. The vapor pressure of ice at the end of the valve closure period (P_{end}) is estimated by Equation 5.

$$P_{end} = P_{ice} + X \cdot t \qquad (5)$$

where P_{ice} is the vapor pressure of ice before valve closure and $X \cdot t$ is "term 3" in the MTM equation. Since pressure rise due to leakage is negligibly small (<0.04 mTorr per second), "term 3" is due almost entirely to the increase in ice temperature caused by heat transfer from the shelf. Therefore, the product temperature at the end of valve closure can be calculated using Equation 3 and Equation 5. Both calculated and experimental product temperature increases during MTM data collection are presented in Table 1. Good agreement between experimental and calculated temperature increases is observed. The results show that the product temperature increased ~1°C during a typical freeze-drying process with a data collection period of 25 seconds, and it increased more than 2°C in an aggressive freeze-drying process (shelf temperature +20°C). The "typical" freeze-drying process refers to freeze-drying of 5% sucrose at a shelf temperature of −20°C and chamber pressure of 60 mTorr. It is obvious from Equation 5 that the vapor pressure of ice at the end of the valve closure period increases linearly with valve closure time, and the longer the valve closure time the larger the product temperature increase. Thus, increasing the MTM data collection time in

Table 1. Experimental and Calculated Increases in Product Temperature (T_p) During a 25-Second MTM Procedure for Vials Containing (~0.7 cm) 1 g Ice*

Formulations	Chamber Pressure/mTorr	Shelf Temperature/°C	T_p/°C	Calculated T_p Increase/°C	Experimental T_p Increase/°C
5% sucrose	60	−20	−36	0.7	1
5% glycine	80	20	−25	2.1	2

*MTM indicates manometric temperature measurement.

order to ensure an adequate temperature-dominated phase for low Q value products (lower than 0.2) is not practical.

The MTM data collection is usually no longer than 25 seconds, so the Q value of 0.23 (with an exponential phase of 20 seconds) is about the minimum value for an accurate MTM product temperature determination. If the Q ratio is much less than 0.2, then the product temperature-dominated phase will occur too late for the MTM measurement to be effective. For example, at a Q ratio of 0.05, which is the case for freeze-drying 12 vials of 5% glycine, it will take more than 250 seconds to allow the product temperature-dominated phase to appear.

The Effect of Ice-sublimation Area: Experimental Results

Four freeze-drying experiments were performed with 5% glycine using a different number of vials (ie, 4, 12, 50, and 150 vials) in each trial. Thus, different ice-sublimation areas were used, (corresponding to the ice sublimation areas of 12, 35, 146, and 437 cm^2, respectively) at a shelf temperature of −20°C and chamber pressure of 80 mTorr. MTM and thermocouple product temperatures were in good agreement for 150 and 50 vials (Figure 4A and B). However, the MTM product temperatures, T(MTM), were much lower than thermocouple product temperatures, T(TC), for the small ice-sublimation area experiments (ie, 12 and 4 vials) even though the data were well represented by the MTM equation (ie, the regression analysis converged to give a good fit) (Figure 4C and D). Therefore, convergence and a good fit are not a sufficient condition for an accurate MTM product temperature, and existence of the temperature-dominated phase is required for accurate MTM product temperature measurement. The experimental results support the simulation results in that the temperature-dominated part will not appear if the number of vials (5 mL) is less than ~50. That is, for a freeze-dryer with a chamber volume of 52 L, there is a minimum ice-sublimation area of 150 cm^2 (50 vials) to permit accurate MTM product temperature, which corresponds to a minimum Q value of ~0.2.

Impact of Product Temperature Heterogeneity on the MTM Procedure

Magnitude of product temperature heterogeneity

The product temperatures are location dependent.[3] The front vials have the highest product temperature, the central vials have the lowest, and the side and back vials have product temperatures that fall in between (Table 2). For example, in experiment No. 6, the product temperature was −37°C at the center, which is 4°C lower than the front and 1°C lower than the side. In experiment No. 1, the temperature differences were even larger. Here, the central vials had a product temperature 7°C lower than front corner vials and 3.5°C lower than the front vials. When 5% glycine was freeze-dried at a shelf temperature close to ambient (experiment No. 2), the product temperature heterogeneity was greatly reduced. In this experiment, the product temperature difference between internal vials and front vials was ~2°C. Some temperature bias remained because the internal

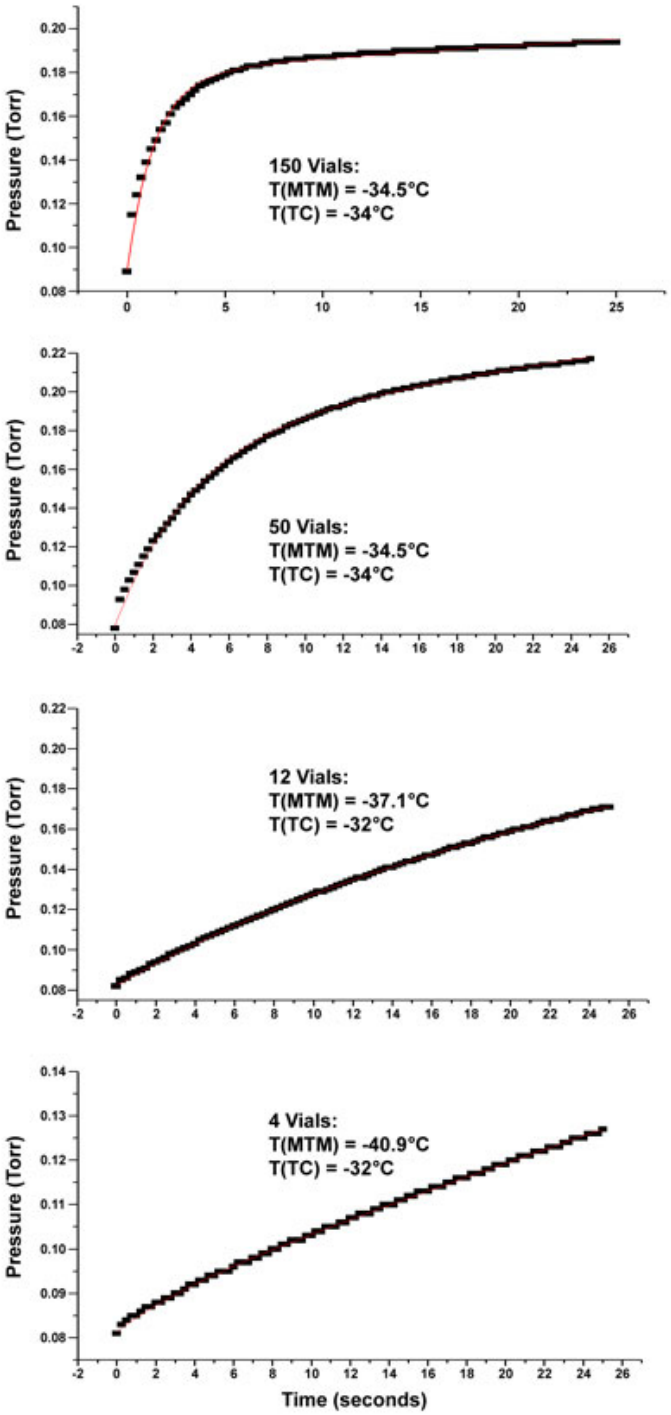

Figure 4. Fit of MTM equation to experimental pressure rise data for different loads: 5% glycine freeze-dried at shelf temperature of −20°C and chamber pressure of 80 mTorr. The symbols are raw experimental data and the line is from MTM fit.

Table 2. Freeze-drying Product Temperature Heterogeneity Under Different Freeze-drying Conditions*

Experiment No.	Product	Thermal Shields	T_s/ °C	TC Center/ °C	TC Side/ °C	TC Front/ °C	TC Front Corner/ °C	MTM/ °C
1	5% glycine	no shields	−20	−33.4	−30.3	−29.5	−26.4	−33.5
2	5% glycine	no shields	20	−26.0	−25.0	−24.0	-	−25.5
3	5% glycine	dummy vials	−20	−34.4	−31.7	−30.1	-	−33.8
4	5% glycine	aluminum foil	−20	−34.1	−32.1	−31.0	-	−34.3
5	5% glycine	dummy vials and alluminum foil	−20	−34.0	−31.6	−31.4	-	−34.0
6	5% sucrose	no shields	−30	−37.2	−36	−33	-	−37.4

*T_s indicates shelf temperature; TC, thermocouple product temperature; and MTM, manometric temperature measurement. Product temperature measurements were performed in the middle of primary drying, and the thermocouple responses were corrected for the temperature difference (ΔT) across the frozen layer. Chamber pressure = 80 mTorr.

vials still viewed other low temperature sample vials (about −25°C), while the edge vials viewed the ambient temperature walls, which have a surface temperature much higher than that of samples (~20°C). Thermal shields (ie, dummy vials and/or aluminum foil attached inside the freeze-drying chamber door) reduced radiation heat transfer from the chamber door and walls to sample vials and reduced the product temperature difference between internal and edge vials (Table 2). The thermal shields are an effective means of minimizing this unfavorable effect, although they could not completely remove the product temperature heterogeneity.

Product temperature heterogeneity is unfavorable for freeze-drying because it complicates the freeze-drying process design and extends the process. A well-optimized freeze-drying process is designed to operate at a specific target product temperature, which is only several degrees lower than the collapse temperature of the formulation. The heat input has to be adjusted so that even the warmest vials (the edge vials) are below the collapse temperature. However, the duration of freeze-drying is determined by the coldest vials (central vials). Thus, the shelf temperature is decreased to avoid collapse in the edge vials but the process then runs much longer to ensure the central vials are dry. Therefore, a longer freeze-drying cycle is expected for freeze-drying with higher product temperature heterogeneity.

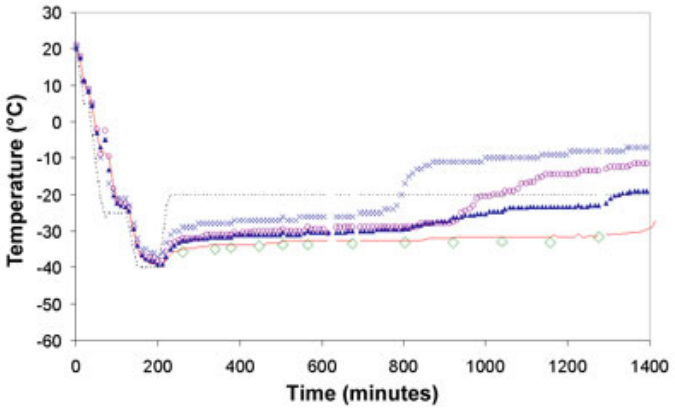

Figure 5. MTM product temperatures in a heterogeneous temperature system. MTM measures a temperature close to the coldest temperature in the system. Freeze-drying of 5% glycine with a load of 150 vials, 2 mL fill. T_s = −20°C, P_c = 80 mTorr, and no thermal shields were used. ◇ = MTM temperature; ○ = front temperature; ▲ = side temperature; — = center temperature; – = shelf temperature; and × = front corner temperature. Thermocouple temperatures are temperatures at the bottom of the frozen layer.

What kind of "average" product temperature is measured by MTM?

It was shown (Table 2, experiments Nos. 1 and 2) that a higher shelf temperature produces higher product temperature and less heterogeneity in product temperature. Moreover, the MTM product temperatures matched most closely product temperatures in the internal vials, which were the lowest product temperatures for both conditions. Indeed, all experiments (Table 2) show that the MTM product temperatures were consistent with the temperature of the internal vials. Figure 5 compares the MTM product temperature and individual vial temperatures measured with thermocouples during the entire primary drying process. Here, we observed very large product temperature heterogeneity with the higher temperature vials freeze-drying much faster than

Table 3. Product Temperatures in a Mixed Product Load of 138 Vials of 5% Glycine and 12 Vials of Pure Water Freeze-dried at Shelf Temperature of −20°C and Chamber Pressure of 80 mTorr*

Product	Number of Vials	Temperature °C, TC	Temperature °C, MTM
H_2O	12	−39	-
5% glycine	138	−31	-
Average	-	−31.6	−38.5

*TC indicates thermocouple; and MTM, manometric temperature measurement.

the lower temperature vials and, most important, the MTM product temperatures were again close to the product temperature measured in the center vials. Correction of the thermocouple data for the temperature gradient across the frozen layer would result in near perfect agreement.

The next question we addressed was whether or not the MTM temperature corresponded best with the interior vials because they were the most numerous or because they were the coldest. In order to answer this question, a special freeze-drying experiment was constructed such that the low product temperature vials were far fewer in number than the high product temperature vials. A mixture of 138 vials of 5% glycine and 12 vials of pure water were freeze-dried at a shelf temperature of −20°C and chamber pressure of 80 mTorr. Here, there are 138 higher temperature (−31°C) vials compared with 12 lower temperature (−39°C) vials. The results showed that the MTM product temperature (−38.5°C) was still close to the lowest product temperature (ie, −39°C for the vials of water) even though there were far more higher product temperature (−31 °C) vials in the dryer (Table 3). In fact, the pressure in the freeze-drying chamber during the pressure rise procedure never reached the value of the vapor pressure of ice in the glycine vials (231 mTorr at −31°C) although the pressure rise, P(t) in Equation 1, is typically above P_{ice} at the later stage of the valve closure. This observation strongly suggests that the water vapor from the high temperature ice (glycine samples) condenses onto the low temperature surface of pure ice vials during the period of MTM data collection, thereby giving a temperature via MTM close to that of the cold vials. Thus, the MTM method measures "average" product temperature heavily weighted in favor of the coldest temperature (Table 3). Note that in a normal freeze-drying operation, the colder vials (ie, interior vials) are by far the most numerous, therefore MTM usually measures the "number average" temperature. Typically, the high product temperature location in a freeze-drying run is a concern for product quality. Such location variations are normally more severe for a laboratory freeze-dryer than for a manufacturing freeze-dryer since the emissivities of door and walls are significantly less for a manufacturing dryer (ie, highly polished stainless steel on all surfaces). Still, even in manufacturing, it is always a good practice to have a "safety margin" between the target product temperature and the product collapse temperature and/or Tg'.[11,12]

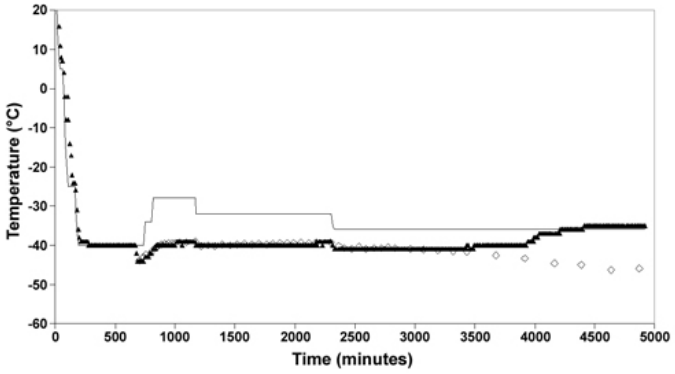

Figure 6. Comparison of MTM product temperatures with thermocouple temperatures for freeze-drying at low product temperature: Freeze-drying of 5% glycine at P_c = 50 mTorr. ◇ = MTM product temperature; ▲ = thermocouple product temperature; — = shelf temperature.

The Low Temperature Limit for MTM

To test the accuracy of MTM at very low temperature, freeze-drying of 5% sucrose was performed at a target product temperature of −40°C. Product temperatures measured by both MTM and thermocouples are illustrated in Figure 6. Note that MTM and thermocouple product temperatures were in excellent agreement throughout nearly all of the primary drying, with deviations only occurring after much of the load ends primary drying. The temperature gradient across the frozen solution was negligible in this freeze-drying experiment (<0.1°C), so the thermocouple temperature could be directly compared with MTM product temperature, without correction for the temperature gradient in the frozen layer. Note that the product temperature at the very beginning of primary drying was −45°C (Figure 6), which is about as low as encountered in any practical freeze-drying run, and yet MTM and thermocouple data are in excellent agreement. Thus, the earlier speculation[7] suggesting that the MTM procedure would not work below about −35°C was far too pessimistic.

CONCLUSIONS

There is a minimum ice-sublimation area requirement for accurate MTM product temperature measurement. For a typical laboratory freeze-dryer with a 50-L chamber volume, the minimum ice-sublimation area required is 150 cm^2 or a minimum of 50 5-mL vials. The minimum ice-sublimation area can be estimated by the Q value (in general, $Q \geq 0.23$). The product temperatures during freeze-drying are location dependent, with higher temperatures occurring for edge vials and lower temperatures for internal vials. The exact product temperature heterogeneity is specific to the freeze-drying conditions, which can be minimized by applying thermal shields (ie, empty vials around the sample vials and aluminum foil attached to the inside of the chamber door). In a system heterogeneous in product temperature, MTM measures a temperature close to the coldest temperature in the system. Finally, MTM provides a valid measurement of product temperature during primary drying even at temperatures as low as −45°C.

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REFERENCES

1. Pikal MJ. Lyophilization. In: Swarbrick J, Boylan J, eds. *Encyclopedia of Pharmaceutical Technology.* New York, NY: Marcel Dekker, 2001; 1299–1326.

2. Carpenter JF, Pikal MJ, Chang BS, Randolph TW. Rational design of stable lyophilized protein formulations: some practical advice. *Pharm Res.* 1997;14:969–975.

3. Pikal MJ, Roy ML, Shah S. Mass and heat transfer in vial freeze-drying of pharmaceuticals: role of the vial. *J Pharm Sci.* 1984;73:1224–1237.

4. Pikal MJ. Use of laboratory data in freeze-drying process design: heat and mass transfer coefficients and the computer simulation of freeze-drying. *J Parenter Sci Technol.* 1985;39:115–139.

5. Pikal MJ. *Freeze-drying of proteins: process, formulation, and stability. ACS Symp Series*. Washington, DC: American Chemical Society; 1994:120–133.

6. Roy ML, Pikal MJ. Process control in freeze-drying: determination of the end point of sublimation drying by an electronic moisture sensor. *J Parenter Sci Technol.* 1989;43:60–66.

7. Milton N, Pikal MJ, Roy ML, Nail SL. Evaluation of manometric temperature measurement as a method of monitoring product temperature during lyophilization. *PDA J Pharm Sci Technol.* 1997; 51:7–16.

8. Pikal MJ. Freeze-drying of proteins. II. Formulation selection. *BioPharm.* 1990;3:26–30.

9. Pikal MJ, Shah S, Senior D, Lang JE. Physical chemistry of freeze-drying: measurement of sublimation rates for frozen aqueous solutions by a microbalance technique. *J Pharm Sci.* 1983; 72:635–650.

10. Tang XC, Nail SL, Pikal MJ. Mass transfer in freeze-drying: Measurement of dry layer resistance by a non-steady state method (the MTM procedure). *AAPS PharmSci (supplement).* 1999;S-564 Abstract 3740.

11. Tang XC, Nail SL, Pikal MJ. Freeze-drying process design by manometric temperature measurement: design of a smart freeze-dryer. *Pharm Res.* 2005;22:685–700.

12. Tang X, Pikal MJ. Design of freeze-drying processes for pharmaceuticals: practical advice. *Pharm Res.* 2004;21: 191–200.