The Effect of Tertiary Butyl Alcohol on the Resistance of the Dry Product Layer During Primary Drying

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The addition of low concentrations of tertiary butyl alcohol (TBA) (3–10% w/v) was shown to influence the crystal habit of ice by causing the formation of needle-shaped ice crystals. The sublimation of these needle-shaped ice crystals resulted in a dry product layer with little resistance. Using a microbalance technique it was shown that the primary drying stage for 5% w/v sucrose solution was considerably shorter when 5% w/v TBA was added due to a lower resistance of the dried cake above the frozen solution. In the absence of TBA the frozen solution had an initially high resistance of approximately 60 cm^2 torr hr/gm due to the formation of a skin; once the skin cracked the resistance reached a constant value of 10 cm^2 torr hr/gm. The solution containing TBA had a dried product resistance in the range of 0.5–3 cm^2 torr hr/gm. The total time required to dry the product in the absence of TBA in the microbalance was 100 hours as compared to 10 hours for the solution containing 5% w/v TBA. The specific surface area of the freeze-dried cake produced from a 5% w/v sucrose solution containing 5% w/v TBA was 8.57 m^2/gm as compared to 0.67 m^2/gm for solutions not containing TBA. The addition of TBA to the sucrose solution did not change the collapse temperature, but the rapid rate of sublimation prevented the product from ever reaching the collapse temperature.

KEY WORDS: lyophilization; tertiary butyl alcohol; collapse temperature; primary drying; freeze-drying microbalance.

INTRODUCTION

Freeze-drying involves the removal of water from a frozen solution by sublimation. Many products freeze-dry in an unacceptable manner showing evidence of cavitation, collapse, puffing, or foaming. To avoid these occurrences, lower drying temperatures are often employed, a practice which leads to long drying cycles. The economic consequences become an issue when extremely long cycles are used. This issue becomes even more problematic if the freeze-dried product has to be discarded due to substandard quality. Ideally, manufacturers would like to accelerate the freeze-drying process while at the same time obtain a pharmaceutically acceptable product.

Freeze-drying has gained increasingly more utilization for the preservation of biopharmaceutical products. Often during the freeze-drying of proteins, sugars and other polyhydroxy compounds are added to serve as lyoprotectants (2). Generally, these protective agents must at least remain partially amorphous to adequately protect the protein during freezing and drying (3,4). While these sugars and polyhydroxy compounds serve to protect the protein, they tend to pose a problem for the freeze-drying process, because these amorphous systems have low collapse temperatures. The high cost of the protein products and the fact that the excipients used in the formulation typically have low collapse temperatures dictates the use of slow and sometimes ultraconservative freeze-drying cycles.

These slow and conservative cycles can be optimized by improving the rate of mass transfer of water through the partially dried layer. Mass transfer is usually expressed in terms of resistance with the dried product layer being responsible for almost 90% of the total resistance (5). As drying proceeds, the dried layer becomes thicker and the resistance increases while the heat flux from the shelf remains constant. Eventually a point is reached where the rate of heat-transfer from the shelves exceeds the rate of mass-transfer of water through the partially dried layer. Due to the increase in the vapor pressure of water through the partially dried layer, the temperature of the product increases and hence the chance for a eutectic meltback or collapse increases. It has been shown that the porous texture of the partially dried layer plays an important role in mass-transfer (6). Therefore controlling the physical characteristics of the partially dried layer is the best approach for reducing long freeze-drying cycles. Gatlin, et. al. have shown that cefazolin sodium, when thermally treated, can be transformed from the amorphous form to the crystalline form (7). Such transformation of cefazolin sodium has also been achieved by the addition of isopropyl alcohol (8). By altering the physical characteristics of the partially dried layer, the authors were able to freeze-dry cefazolin sodium at a much higher temperature without collapse.

It has been shown that in the presence of tertiary butyl alcohol (TBA), a low molecular weight alcohol, the drying process can be accelerated, while at the same time obtaining a desirable freeze-dried cake (9). Concentrated sucrose solutions, as high as 30% w/v, were freeze-dried within 24 hours at a shelf temperature of +30°C and a chamber pressure of 100 mTorr (5 ml fill in 20 ml type I borosilicate vials), without any sign of collapse in the presence of 5% w/v TBA. The purpose of this investigation was to assess the value of TBA as a mass-transfer accelerator in freeze-drying. The sublimation rates for frozen aqueous sucrose solutions were studied as a function of thickness of the dried product and the addition of TBA by the microbalance technique (10). The freeze-drying microscope was used for examining the frozen structure of these systems.

EXPERIMENTAL

Materials

Sucrose (J.T. Baker, Philipsburg, NJ), tertiary butyl alcohol (Fisher Scientific, Fairlawn, NJ), freeze-drying microscope (Eli Lilly & Co.), microbalance (Eli Lilly & Co.), surface area analyzer ASAP 2000 (Micromeritics Instrument Corporation, Norcross, GA), krypton gas (99.995 mole %,

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Central Welding Supplies, Inc., Lexington, KY), scanning electron microscope model S-800 (Hitachi). The water used was water for injection (Eli Lilly & Co.).

Freeze-Drying Microscope

The direct observation of the freeze-drying process was accomplished by a freeze-drying microscope. This instrument consists of a high vacuum cold stage used in conjunction with an optical microscope. A drop of the solution was placed on a 24 × 24 mm coverslip and an 18 × 18 mm coverslip was placed on the droplet, causing the solution to spread evenly. Silicone stopcock grease was applied to the bottom of the 24 × 24 mm coverslip to allow for better thermal contact between the coverslip and the temperature-controlled plate. A temperature probe was also glued to the coverslip with the silicone grease. The temperature of the cold stage was controlled by passing cold nitrogen through the channels in the plate. The sample was frozen at a rate of about 1°C/min to −45°C. The sample was then heated to room temperature at a rate of about 1°C/min and thermal events were observed by the microscope.

Measurement of Dried Product Resistance by Microbalance Technique

The dried product resistance was measured using the technique described by Pikal *et al.* (10). A 5% w/v sucrose solution was prepared and about 13 μl was placed in the sublimation cell of the microbalance sublimation apparatus using a syringe. The sublimation cell was suspended from the microbalance which was enclosed by the balance tube. The sample solution was frozen by immersing the balance tube in a dewer containing 2-propanol at −40°C. Once the sample froze and vacuum was applied, the dewer was removed and the balance tube was immersed in a thermostat at a temperature of −35°C. The rate of sublimation was determined from mass loss as a function of time data. A similar experiment was conducted using a 5% w/v sucrose solution containing 5% w/v TBA.

Surface Area Measurements of Freeze-Dried Materials

The specific surface area of the freeze-dried material was determined using a Micromeritics ASAP 2000 (Accelerated Surface Area and Porosity). The measurement was based on the adsorption and desorption of gas (typically nitrogen and krypton) at the surface and within the pores of the solid sample (11). For measuring the surface area of freeze-dried sucrose, krypton gas was used. The low surface area of the material dictated the use of krypton as the adsorbent gas because of its low saturation pressure (12).

Scanning Electron Microscopy

A model S-800, Hitachi scanning electron microscope was used to examine the surface morphology of the freeze-dried sample. A cross-section of the freeze-dried cake was cut and glued to the sample mount. The freeze-dried sample was then coated with conductive gold palladium before scanning.

RESULTS AND DISCUSSION

Resistance of the Dry Product Layer

In the presence of 3–10% TBA needle-shaped ice crystals form during freezing (13). The influence of these needle-shaped ice crystals on the rate of freeze-drying was assessed by measuring the resistance of the dry product layer formed during freeze-drying in a microbalance (10). Figure 1 is the plot of the normalized dried product resistance (R_p) versus the thickness of the dried product layer (L) for a 5% w/v sucrose solution freeze-dried at a temperature of −35°C. A high product resistance of 60 cm^2 torr hr/gm was observed initially and was followed by a sharp decrease in resistance at low L (<0.2 cm of dried product). After the sharp decrease, the dried product resistance leveled off at about 10 cm^2 torr hr/gm. The initial high resistance may be due to an impermeable skin which sometimes forms during freezing. During drying the skin shrinks and cracks. The presence of these cracks allows for better mass-transfer of water vapor, hence the lower dried product resistance. Solutions of sucrose and polyvinyl pyrrolidone have been shown to crack upon prolonged freeze-drying at low temperatures (14).

The mass loss of water versus time is shown in Figure 2. During the first 40 hours of drying, there was no substantial loss of water because of the presence of the impermeable skin at the surface of the frozen sucrose solution. After 40 hours the rate of water loss reached a constant 0.15 mg/hr; this coincided with the moment the dried product resistance-decreased due to the formation of cracks in the amorphous skin. The rate of water loss followed zero-order kinetics.

Figure 3 is the plot of the normalized dried product resistance (R_p) versus the thickness of the dried product (L) for a 5% w/v sucrose solution freeze-dried with 5% w/v TBA at a shelf temperature of −35°C. The normalized dried product resistance increased almost linearly from 0.5 to 3 cm^2 torr hr/gm as the dried product went from 0 to 1.0 cm. The sucrose solution containing TBA formed a dried product layer which had a significantly lower resistance to the mass-transfer of water vapor than sucrose freeze-dried without TBA. MacKenzie, through direct microscopic observation, has divided freeze-drying into six categories, one of which is "water vapor escaping through open channels" (14). The linear increase of the resistance with increasing dried prod-

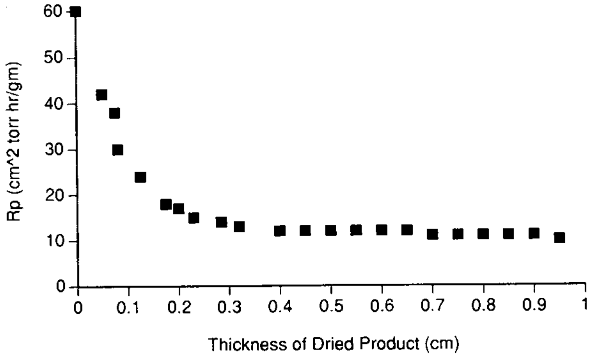

Fig. 1. Normalized dried product resistance versus thickness of dried product (5% w/v sucrose freeze-dried in a microbalance at a temperature of −35°C).

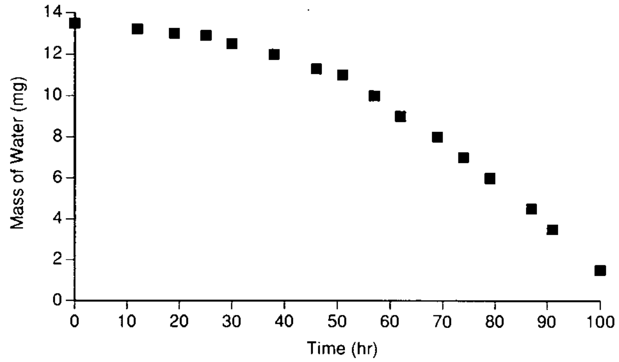

Fig. 2. Mass loss of water from a 5% w/v sucrose solution (drying temperature −35°C, microbalance).

uct thickness is indicative of the "water vapor escaping through open channels" mechanism. The freeze-drying of salt solutions is an example of this type of mechanism. Upon the sublimation of ice from solutions, channels are left behind through which water vapor can flow with little resistance. Figure 4 is the SEM photomicrograph of a 10% sucrose solution freeze-dried in the presence of TBA. The long channels present in the freeze-dried matrix allow for faster drying. Figure 5 depicts the mass loss of water as a function of time for this system. The rate of water loss was 1.25 mg/hr for the sucrose solution containing TBA as compared to 0.15 mg/hr for sucrose solutions without TBA. According to Figure 5, about 12 mg of water sublimed in only 10 hours as compared to 100 hours for sucrose solutions not containing TBA (Fig. 2). The slow rate of vapor flow in systems not containing TBA can be explained from the matrix structure illustrated in Figure 6. Sucrose solutions freeze in a manner which is not favorable to mass transfer.

Effect of TBA on the Collapse Temperature of Sucrose

The collapse temperature of the frozen sucrose solution, with and without TBA, was determined with the freeze-drying microscope. The collapse temperature of sucrose was determined to be −33°C which did not change in the presence of TBA. However, we have freeze-dried 10–30% sucrose solutions containing 5% TBA at a shelf temperature of +30°C and chamber pressure of 100 mTorr (5 ml fill in 10 ml type I borosilicate vials). These samples freeze-dried nicely without collapse because of the faster rate of sublimation. Rapid sublimation in the presence of TBA prevented the collapse of sucrose during freeze-drying for two reasons. First, rapid sublimation resulted in the maintenance of a low product temperature (below the collapse temperature). Second, the water content of the partially dried sucrose layer decreased faster in the presence of TBA so the viscosity increased and hence the viscous flow (collapse) was prevented. Figures 7a and 7b are the polaroid pictures of 5% sucrose without and with 5% w/v TBA, respectively. Region I is the dried sucrose matrix which did not collapse when the temperature was raised to −31°C, because the material was dry enough to avoid viscous flow. However, region II, which is at the interface, did collapse because the moisture content was high enough for viscous flow to occur. Region III, the

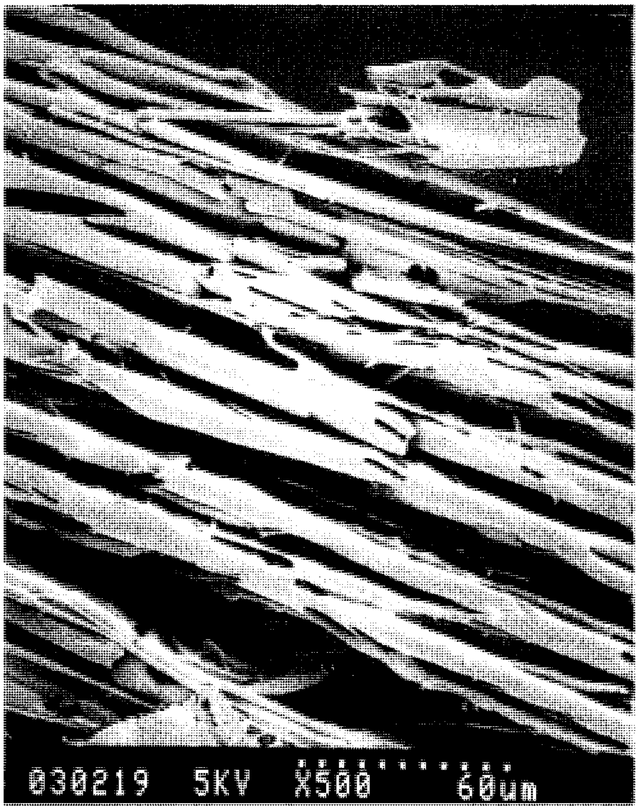

Fig. 4. SEM of sucrose (10% w/v) freeze-dried with TBA. Reproduced at 95% of original.

Fig. 3. Normalized dried product resistance versus thickness of dried product (5% w/v sucrose containing 5% w/v TBA freeze-dried in a microbalance at −35°C).

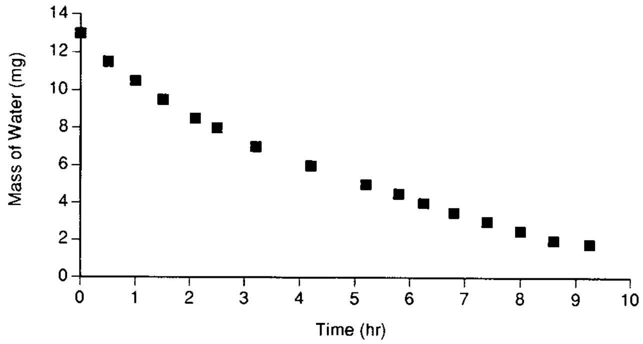

Fig. 5. Mass loss of water from a 5% w/v sucrose solution containing 5% w/v TBA (drying temperature −35°C, microbalance).

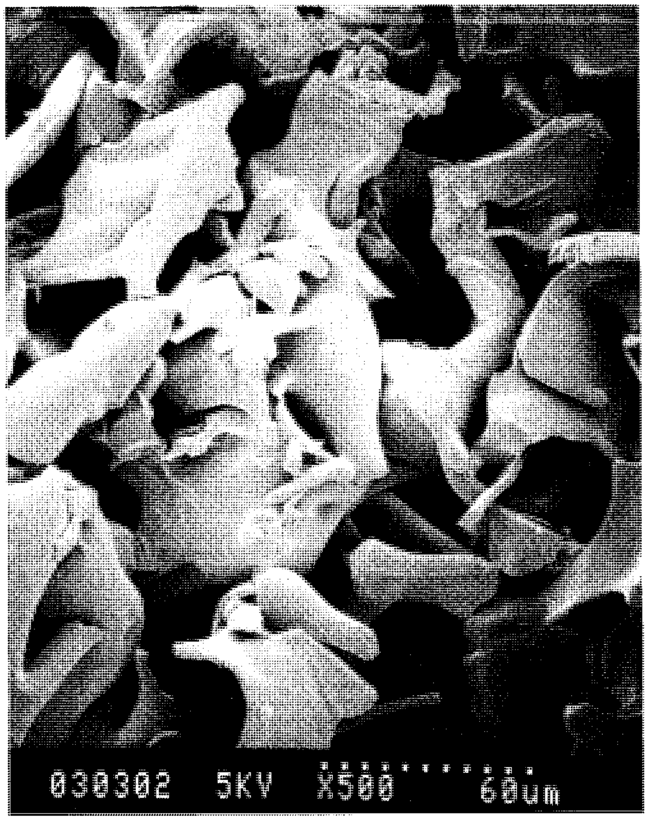

Fig. 6. SEM of freeze-dried sucrose (10% w/v). Reproduced at 95% of original.

frozen matrix, did not collapse because of support from the ice crystals. Figure 7b shows the formation of needle-shaped ice crystals in the presence of TBA.

The Effect of TBA on the Specific Surface Area of the Freeze-Dried Material

The presence of the needle-shaped ice crystals affected the specific surface area of the freeze-dried material. The total surface area of a freeze-dried solute was determined by the surface area of the ice crystals (15). Since TBA alters the crystal morphology of ice, then it must effect the surface area of the freeze-dried cake. The samples freeze-dried without TBA had a specific surface area of 0.67 m^2/gm as compared to a specific surface area of 8.57 m^2/gm for samples freeze-dried in the presence of TBA. The presence of TBA in the 5% sucrose solution resulted in a 13-fold increase in the specific surface area of the freeze-dried material, and hence a faster rate of sublimation. As mentioned earlier, the specific surface area of a freeze-dried product depends on the size and shape of the ice crystals. In the presence of TBA, the water freezes and forms fine needle-shaped ice crystals as opposed to round ice crystals. A mathematical approximation was used to obtain the relationship between the specific surface area and the shape of the ice crystals. The specific surface area of a 5% sucrose solution freeze-dried in the presence of TBA was calculated to be 7.28 m^2/gm. This value was obtained by assuming all of the ice crystals were needle-shaped ($d = 10$ μm, based on SEM observations). The specific surface area of sucrose freeze-dried in the absence of TBA was calculated to be 2.19 m^2/gm. This was

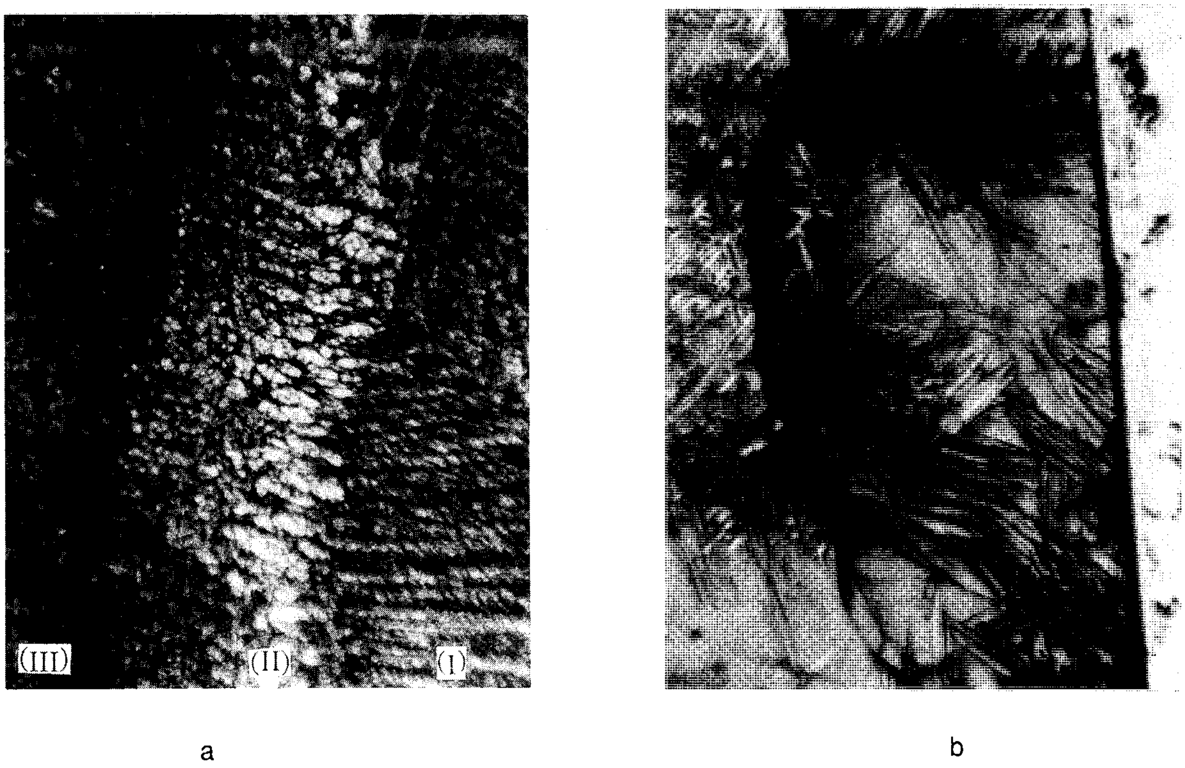

Fig. 7. Polaroid photographs of sucrose during freeze-drying a) Without TBA: Region I: dried material, Region II: collapse, Region III: frozen matrix. b) In the presence of TBA.

calculated assuming that round spherical ice crystals having a diameter of 50 μm formed throughout the matrix.

The calculated specific surface areas agree fairly well with the measured values. Therefore, one can conclude that the formation of fine needle-shaped ice crystals will result in a higher specific surface area than larger spherical ice crystals.

CONCLUSIONS

In the presence of TBA, needle-shaped ice crystals are formed which result in the formation of a low resistant dried product layer during primary drying. The lower product resistance allows for faster rate of sublimation. Rapid sublimation in the presence of TBA prevents the product from reaching its collapse temperature, because the surface concentration of water at the ice-vapor interface will begin to decrease rapidly and the glass transition temperature of the amorphous phase will increase.

The addition of TBA to sucrose resulted in a dried product with a very high specific surface area. The fine needle-shaped ice crystals formed in the presence of TBA have a substantially larger surface area than the larger spherical ice crystals formed when TBA was not present. The higher specific surface area contributes to the faster removal of water.

APPENDIX

A Mathematical Approximation of the Specific Surface Area of 5% w/v Freeze-Dried Sucrose

Assumptions

1. In sucrose solutions containing TBA all of the water freezes as fine needle-shaped ice crystals having a diameter of 10 μm (based on SEM observations).
2. In sucrose solutions not containing TBA all of the water freezes as spherical ice crystals having a diameter of 50 μm (based on SEM observations)
   - The porosity of the freeze-dried cake is 0.97.
   - Fill volume = 5 ml.
   - Depth of liquid = 1.5 cm.
   - Total volume of matrix $= \pi r^2 L = \pi (1)^2 (1.5) =$ 4.71 cc.
   - Volume occupied by ice = porosity × total volume of matrix $= 0.97 \times 4.71 = 4.57$ cc.

Surface Area of Needle-Shaped Crystals

For the purpose of simplifying the calculations, the volume of cylindrical rods were used in the surface area calculations. Volume of a single crystal $= \pi r^2 L = \pi (5 \times 10^{-4}\ \text{cm})^2 L = 7.85 \times 10^{-7} L$. Number of ice crystals = volume of ice/volume of single crystal $= 4.57\ \text{cc}/7.85 \times 10^{-7} L = 5.82 \times 10^6/L$. Surface area of a single ice crystal $= 2 \pi r L = 2 \pi (5 \times 10^{-4}\ \text{cm}) L = 3.14 \times 10^{-3} L\ \text{cm}^2/\text{crystal}$. Total surface area = total number of ice crystals × surface area of one crystal $= (5.82 \times 10^6/L) \times (3.14 \times 10^{-3} L) = 1.82 \times 10^4\ \text{cm}^2 = 1.82\ \text{m}^2/\text{vial}$. Each vial contains approximately 0.25 grams of dried solid. Therefore the approximated specific surface area (S.S.A.) is: approximated S.S.A. $= 7.28\ \text{m}^2/\text{gm}$; Measured S.S.A. $= 8.57\ \text{m}^2/\text{gm}$.

Surface Area of Spherical Ice Crystals

Volume of a single spherical ice crystal $= 4/3\ \pi r^3 = 4/3\ \pi (25 \times 10^{-4})^3 = 6.5 \times 10^{-8}$ cc. Number of spheres $= 4.56\ \text{cc}/6.5 \times 10^{-8}\ \text{cc} = 7 \times 10^7$. Surface area of a single sphere $= 4 \pi r^2 = 4 \pi (25 \times 10^{-4})^2 = 7.85 \times 10^{-5}\ \text{cm}^2/\text{single sphere}$. Total surface area $= (7 \times 10^7)(7.85 \times 10^{-5}) = 5.49 \times 10^3\ \text{cm}^2/\text{vial} = 0.55\ \text{m}^2/\text{vial}$. Approximated S.S.A. $= 2.19\ \text{m}^2/\text{gm}$. Measured S.S.A. $= 0.67\ \text{m}^2/\text{gm}$.

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REFERENCES

1. A. P. MacKenzie. Collapse during freeze-drying qualitative and quantitative aspects. In S. A. Goldblith and L. Rey (eds.), *Freeze Drying and Advanced Food Technology*, Academic Press, London, 1975, pp. 277–307.
2. M. W. Townsend and P. P. DeLuca. Use of lyoprotectancts in the freeze-drying of a model protein, ribonuclease A.. *J. Parent. Sci. Technol.* 42, 190–199 (1988).
3. M. J. Pikal. Freeze-drying of proteins. Part II: Formulation selection. *Biopharm* 3, 26–30 (October 1990).
4. K. Izutsu, S. Yoshioka, Y. Takeda. The effects of additives on the stability of freeze-dried β-galactosidase stored at elevated temperature. *International Journal of Pharmaceutics* 137–146, 1991.
5. M. J. Pikal. Freeze-drying of proteins. Part I: Process design. *Biopharm* 3, 18–27 (September 1990).
6. D. Simatos and G. Blond. The porous texture of freeze-dried products. In S. A. Goldblith and L. Rey (eds.), *Freeze-Drying and Advanced Food Technology*, Academic press, London, 1975, pp. 3–16.
7. L. Gatlin and P. DeLuca. A study of phase transitions in frozen antibiotics by differential scanning calorimetry. *J. Parenteral Drug Association*. 34, 398–403 (1980).
8. Y. Koyoma, M. Kamat, De Angelis, R. Srinivasan, and P. P. DeLuca. Effect of solvent addition and thermal treatment on freeze-drying ofcefazolin sodium. *J. of Parenteral Science and Technology* 42, 47–52, (1988).
9. M. S. Kamat, Ph.D. Dissertation, University of Kentucky, College of pharmacy (1989).
10. M. J. Pikal, S. Shah, D. Senior, J. E. Lang. Physical chemistry of freeze-drying: Measurement of sublimation rates for frozen solutions by a microbalance technique. *J. of Pharmaceutical Sciences* 72, 635–650 (1983).
11. Application Note Number 54, Micromeritics. Surface area analysis. (1988).
12. Application Note Number 18, Micromeritics. Argon/Krypton adsorption on low surface area materials using Flowsorb II 2300. (1992).
13. K. Kasraian and P. P. DeLuca, Thermal analysis of the tertiary butyl alcohol-water system and its implications on freeze-drying. *Pharm. Res.* 12, 484–490 (1995).
14. A. P. MacKenzie. Factors affecting the mechanism of transformation of ice into water vapor in the freeze-drying process. *Ann. N.Y. Acad. Sci.* 125, 522 (1965).
15. M. J. Pikal, and S. Shah. The collapse temperature in freeze-drying: Dependence on measurement methodology and rate of water removal from glassy phase. *International Journal of Pharmaceutics* 62, 165–186 (1990).