

Optimizing Freeze Drying for DMSO Solutions

I. Introduction

Dimethyl Sulfoxide (DMSO) solutions are widely used in various research and clinical environments due to their unique properties, such as the capacity to permeate biological membranes and act as a cryoprotectant ^{1,2}. Freeze drying or lyophilization is a vital step for long-term storage and transport of DMSO solutions. However, the process presents challenges, primarily due to DMSO's low freezing point and its tendency to form eutectic mixtures with water ³. For successful lyophilization of DMSO water eutectic mixtures, a freeze dryer with a low temperature collector and temperature-controlled shelves capable of reaching and maintaining low temperatures is essential.

The phase diagram of a DMSO water solution provides essential insights into the eutectic properties, which is crucial for the development of precise freeze drying protocols. Gaining a comprehensive understanding the physical and chemical properties of DMSO water solutions is essential for effectively navigating the complex freeze drying process.

II. Freeze Drying DMSO Solutions Challenges

A 66% DMSO water solution has a freezing point of -73°C , which is a lower temperature than most conventional freeze dryers can achieve⁴. For optimal freeze dry results, it is recommended to maintain a collector temperature that is 15°C to 20°C colder than the sample's freezing point. This low freezing point poses a significant challenge for successful sublimation. If the heat transfer during the sublimation phase is not controlled or the collector is not cold enough, localized regions of the sample may exceed the eutectic temperature, triggering a meltback situation.

This meltback could lead to the formation of a liquid phase at the sample's bottom, which is isolated from the vacuum by layers of partially freeze-dried material. This causes pressure to build up in this region, disrupting uniform drying

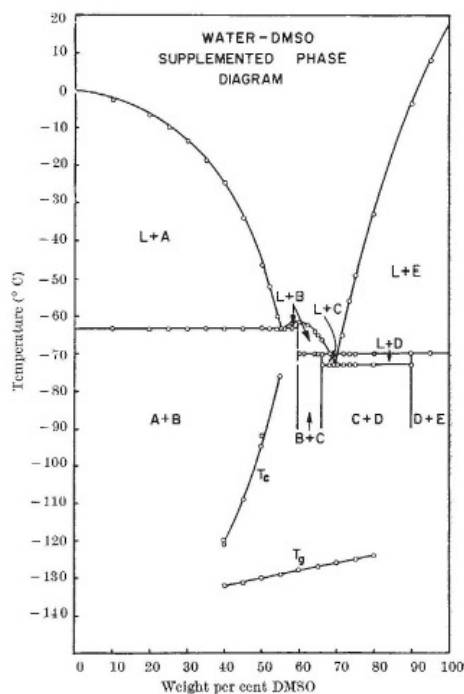


Figure 1: Water-DMSO Phase Diagram from Reference 4

and potentially compromising the integrity of the freeze dried product and the desired lyophilization cake⁵. Pressure build-up also poses a risk of a blow-out: The pressure within the isolated liquid phase can cause a rupture in the overlying freeze dried layer, ejecting the liquid and potentially contaminating other samples in the freeze dryer.

Mitigating meltback and pressure build-up demands precise freeze drying parameters for samples with low freezing point like DMSO. Controlled regulation of sample temperature is essential to ensure efficient sublimation while avoiding localized melting. This emphasizes the advantages offered by freeze dryers equipped with temperature-controlled shelves, such as Labconco's FreeZone® Stoppering Tray Dryers or FreeZone® Triad™ Freeze Dryers.

When freeze drying DMSO solutions using traditional flasks, additional challenges arise. Without temperature control capabilities, samples in flasks cannot be cooled and are exposed to room temperature. These conditions can alter the freeze drying process, leading to drying inconsistencies, sample meltback and compromised product quality. Such issues are particularly detrimental where high product quality and consistency are vital. In situations where flask freeze drying is the only option, specific formulations of DMSO may call for the use of a vacuum concentrator. Vacuum concentration effectively removes DMSO from the sample prior to starting the freeze drying process, optimizing final results. With the DMSO removed, the freezing point of the sample is raised making it less challenging to lyophilize.

Proper handling DMSO in freeze dryers requires extra care to the equipment and attention maintenance. Due to DMSO's low freezing point, it can be challenging to condense all its vapors on the collector. If DMSO vapors bypass the collector, they may reach the vacuum pump and potentially cause damage. It is crucial to avoid overloading the collector and be aware that DMSO vapors can damage both dry and oil-sealed pumps due to the pump's incapability to handle solvents.

To mitigate these risks when utilizing DMSO in a freeze dryer, routine maintenance tasks such as regular defrosting, draining and cleaning of the collector is necessary. Additionally, the vacuum pump oil should be changed regularly, and the pump flushed with clean oil to prevent potential damage. These maintenance tasks are imperative to ensure the longevity of the equipment when subjected to long-term use of DMSO.

III. The Merits of Temperature Controlled Shelves in Freeze Drying DMSO Solutions

Temperature-controlled shelves have an essential role in managing the freeze drying process of DMSO solutions, particularly in maintaining the appropriate balance between temperature and pressure, which are crucial factors in achieving optimal product consistency⁶.

The ability to control the temperature of the shelf allows for optimal freeze drying conditions by maintaining a sufficiently low temperature for sublimating DMSO solutions and avoiding sample meltback. This consistent and controlled environment achieves more uniform drying, shorter drying times and improved product quality⁷. Additionally, Labconco's Triad Freeze Dryer, featuring a temperature-controlled shelf ranging from -55° C to 50° C during operation and -75° C during Max Cold pre-freezing, provides an advanced level of control throughout the lyophilization process of DMSO solutions.

Pairing temperature-controlled shelves with the Universal Tube Bath and Lab Armor Beads accessories can resolve heat transfer issues as well. Tests have demonstrated that when temperature-controlled shelves are set at -40° C while utilizing the Universal Tube Bath, DMSO water mixtures at various concentrations (0.1%, 1%, 10% and 25%) do not experience meltback during the lyophilization process. These same sample concentrations did experience meltback when subjected to flask lyophilization or tray freeze drying without using the Universal Tube Bath and Lab Amor Beads. This is important to consider when freeze drying valuable products.

The DMSO water mixtures were successfully sublimated during the lyophilization process by adhering to specific parameters. This success was achieved by using a Labconco Triad Freeze Dryer, where the vacuum was set to 0.02 mbar for optimized sublimation conditions. The condenser maintained a temperature of -85°C , while the shelf temperature was carefully regulated at -55°C . To further enhance the process, we placed samples inside the Labconco Large Universal Tube Bath with Lab Armor Beads positioned around the samples in the vials. These controlled conditions effectively kept the DMSO water mixture in a solid state, preventing any sample meltback.



Figure 2: Labconco Large Universal Bead Block (4026601) and Lab Armor Beads for Universal Block (4026602) shown inside a FreeZone Triad Freeze Dryer (794001010).

IV. Conclusion

Freeze drying DMSO solutions poses many unique challenges due to the inherent properties of DMSO itself. Flask freeze drying, although common, can introduce variability and inconsistencies due to environmental factors and uneven heat transfer.

Using temperature-controlled tray dryers to freeze dry DMSO solutions, paired with the Universal Tube Bath accessory with aluminum beads, results in a more controlled and efficient process. Incorporating these systems enables precise temperature control and better heat transfer, resulting in enhanced quality and consistency of the final product.

Efficient heat transfer is a crucial step in the freeze drying process, and utilizing Lab Armor Beads helps resolve this issue. These beads facilitate conduction-based heat transfer, ensuring the vials maintain temperatures closer to the shelf temperature. Tests have shown that without the beads, there was a significant difference in temperature with the chamber being 25°C warmer than the shelf when the shelf temperature was set at -55°C .

However, when using a Universal Tube Bath in the same setting, it resulted in a significant improvement of temperature management. The bead block was only 10°C warmer than the shelf when the shelf temperature was set at -55°C , substantially reducing the temperature differential and ensuring the DMSO water mixture could sustain a solid form for successful freeze drying.

In conclusion, the use of advanced, temperature-controlled freeze drying equipment is pivotal in overcoming the challenges of freeze drying DMSO solutions. Through ongoing advancements and a deep understanding of the specific requirements of these solutions, we can enhance the efficiency, consistency, and quality of freeze dried DMSO solutions.

References:

1. Catalán, J., C. Díaz, and F. García-Blanco. "Characterization of binary solvent mixtures of DMSO with water and other cosolvents." *The Journal of organic chemistry* 66.17 (2001): 5846-5852.
2. Kirchner, Barbara, and Markus Reiher. "The Secret of Dimethyl Sulfoxide– Water Mixtures. A Quantum Chemical Study of 1DMSO– n Water Clusters." *Journal of the American Chemical Society* 124.21 (2002): 6206-6215.
3. Idrissi, Abdenacer, et al. "Thermodynamics of mixing water with dimethyl sulfoxide, as seen from computer simulations." *The Journal of Physical Chemistry B* 118.29 (2014): 8724-8733.
4. Rasmussen, D. H., and A. P. MacKenzie. "Phase diagram for the system water–dimethylsulphoxide." *Nature* 220.5174 (1968): 1315-1317.
5. Wong, Daryl B., et al. "Water dynamics in water/DMSO binary mixtures." *The Journal of Physical Chemistry B* 116.18 (2012): 5479-5490.
6. LeBel, R. G., and D. A. I. Goring. "Density, Viscosity, Refractive Index, and Hygroscopicity of Mixtures of Water and Dimethyl Sulfoxide." *Journal of Chemical and Engineering Data* 7.1 (1962): 100-101.
7. Rockinger, Ute, et al. "DMSO as new, counterintuitive excipient for freeze-drying human keratinocytes." *European Journal of Pharmaceutical Sciences* 160 (2021): 105746.

LABCONCO CORPORATION

8811 Prospect Avenue
Kansas City, MO 64132
(800) 821-5525 | (816) 333-8811

labconco.com



©2023 LABCONCO CORPORATION
Design subject to change without notice.
20230623