Adoption of Controlled Ice Nucleation Technology, ControLyo®

From R&D to Commercial Manufacturing



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Abstract

Stabilization by lyophilization is attractive to retain a drug product's potency, structural integrity and homogeneity, all of which are crucial to the success of a pharmaceutical product. Lyophilization begins by lowering the temperature of the product to below its freezing point to form ice crystals (nucleation). However, this process is random and often results in heterogeneity and poor product reconstitution.

This article describes various techniques used to control ice nucleation which have been widely used in research and development environments, and demonstrate its use in production manufacturing. It will focus on one of these methods, ControLyo® which uses a pressurization and depressurization technique to provide simultaneous ice nucleation. The advantages of implementing ControLyo will be discussed including its role in creating a smooth transition between lab- and commercial- scale lyophilization.

Introduction

Controlling reproducibility and inter-/intra- batch variability are key objectives of the manufacturing process for all drugs but particularly in the case of parenteral drugs. Lyophilization is part of this process which stabilizes a drug for storage and transport but requires retention of its potency, structural integrity and homogenous quality. To fully control the freeze-drying process, it is important that each stage of lyophilization and the individual products are thoroughly understood and optimized while, at the same time considering future upscaling of product manufacturing from the R&D laboratory to commercial production.

Ice Nucleation

Successful freeze-drying starts with the initiation of the freezing phase (ice nucleation). Ice nucleation is a stochastic (random and uncontrolled) process which makes it challenging to produce a uniform batch of lyophilized products of consistent quality. The contents of individual vials often nucleate over a broad range of temperatures which can span between 10–15°C below the formulation's thermodynamic freezing point in a laboratory freeze dryer and even greater in a manufacturing cleanroom environment. Vials that nucleate at separate temperatures which will strongly influence the resulting ice crystal size, will dry at various rates and have different pore structure, cake morphology and specific surface area. A high degree of supercooling (colder nucleation temperature) leads to small ice crystals, which in turn results in small pores of high resistance in the product and slower sublimation rates (Figure 1).

To overcome limitations, various methods and technologies have been investigated to initiate nucleation and optimize the drying process. The most common of these include the addition of an **annealing cycle** or the use of a **controlled nucleation technique**, such as pressurization and depressurization technique.

Adding an **annealing cycle** to maintain the formulation above its sub ambient glass transition temperature (Tg'), and below the ice melting temperature for a specific length of time, has been the standard method for many years to create larger ice crystals and minimize the fluctuation in drying rates (Figure 2)[1]. However, this technique can be detrimental to protein stability and doesn't standardize the nucleation temperature which can still result in batch variability.

There are less common alternative approaches to create nucleation, however many of these options are only feasible at the laboratory-scale production of lyophilized samples and are not possible for larger-scale commercial production. Although one of these methods (ice-fog technique) controls nucleation, the vials generally nucleate within a minute or two from each other rather than simultaneously since distribution of the ice-fog in the convoluted freeze dryer chamber can be problematic.

The **pressurization/depressurization** technique of controlled ice nucleation involves cooling the entire batch of vials to a temperature below the thermodynamic freezing point but above the temperature at which stochastic nucleation starts, followed by pressurization of the freeze-dryer chamber with an inert gas such as nitrogen or argon. When thermal equilibrium has been achieved, the excess pressure is released rapidly (depressurization), causing ice crystals to form at the top of the solution and propagate throughout the vial within seconds. With this method, ice formation is induced at virtually the same time for all vials in the batch (Figure 3).

Benefits of Controlled Ice Nucleation (CIN) Technology

One pressurization/depressurization technique that produces homogeneity of vials and between batches during the lyophilization process is ControLyo from SP Scientific. ControLyo utilizes an inert gas and a series of pressurization and rapid depressurization steps to create instantaneous ice nucleation in all vials in the product chamber at a higher temperature. This minimizes supercooling and yields the largest possible ice crystals. Large crystals generate bigger cavities as the ice sublimes resulting in less resistance for subsequent drying of internal areas and the shortest possible drying time.

Some of the studies using CIN have demonstrated many benefits to the lyophilization process:

1. Decreased cycle time - Structural integrity of a biologic drug product is reliant on a slow, gradual drying process which can take several days to complete. ControLyo enables controlled ice nucleation to occur at warmer temperatures which creates larger ice crystals with reduced resistance to mass transfer and decreases drying time. Previous studies have shown that for every 1°C increase in nucleation temperature, the primary drying time is reduced by 3% [2]. In some instances, ControLyo has been shown to reduce the cycle time from 7.5 to 5.5 days which increases productivity and provides economic benefits (unpublished data).

2. Reduced vial breakage - In the absence of annealing, some products can randomly transition through crystalline phases during the drying process, resulting in vial cracking. Vial breakage is costly and disruptive but can be substantially mitigated by using ControLyo technology which induces nucleation at warmer temperatures. In collaboration with a large Pharmaceutical company, it has been demonstrated that lyophilization of a small molecule drug was improved with controlled nucleation occurring at a higher temperature of -4 to -6°C with no cracked vials compared to uncontrolled nucleation at -16 to -19°C with 14% cracked vials. The cake appearance was also considerably improved and consistent.

3. Product quality - Each part of the freeze-drying process has a significant impact on product quality, appearance and uniformity. *Consistency* within and between batches is an integral quality parameter. Variation among vials with ControLyo technology is minimal because all vials freeze at the same product temperature. Defects in *cake appearance*, are caused by suboptimal freezing. Controlled nucleation with ControLyo can adjust freezing behavior to optimize cake elegance. The *yield* of the product can also be improved by controlled nucleation. Protein aggregation, which can reduce product yield tends to occur on the surface of ice crystals. However, the larger crystals associated with ControLyo have a reduced surface area and ControLyo users have reported a reduction in protein aggregation with increased product yield.

4. Maintained drug potency – The success of the freeze-drying process can be measured by the end-product, which in many cases would be a drug product. In the case of biologic drugs, maintenance of drug activity is particularly challenging. Increased reproducibility, repeatability and homogeneity are good indicators of optimal lyophilization and are all improved with controlled ice nucleation, such as using ControLyo technology. To demonstrate the effect of controlled nucleation on drug activity, a research study examined protein degradation with respect to freeze-drying heterogeneity[3]. The study compared CIN using ControLyo and annealing processes for their impact on sodium phosphate buffer crystallization and degradation of β-galactosidase. Annealed lyophilized formulations showed 12–40% loss of activity. In contrast, CIN formulations showed 9–14% loss of activity.

5. Reduced reconstitution time – Increased time for reconstitution could be detrimental to the patients, particularly in emergency situations. Therefore, knowledge of intrinsic and extrinsic parameters that influence reconstitution time and reproducibility are important performance factors for lyophilization. For most formulations, reconstitution time is shorter for products lyophilized with CIN, such as ControLyo. This is thought to be due to larger pores created with higher ice nucleation temperatures.

SEM (Scanning Electron Microscopy) Images of Sucrose, 75 mg/mL, Processed:

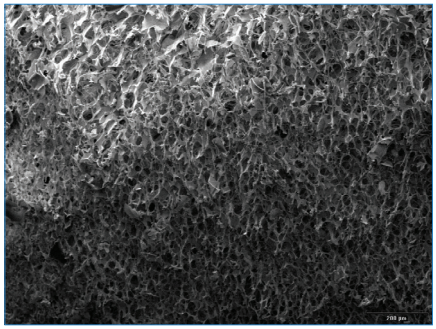


Figure 1: *Using 1°C/min shelf cooling rate ("uncontrolled")*

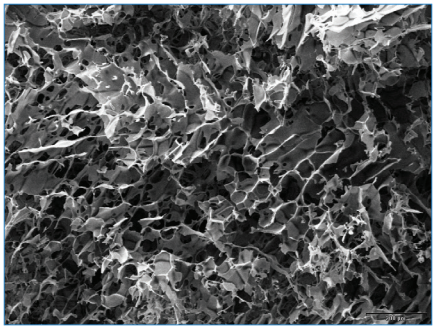


Figure 2: *Using 1°C/min shelf cooling rate plus annealing ("anneal")*

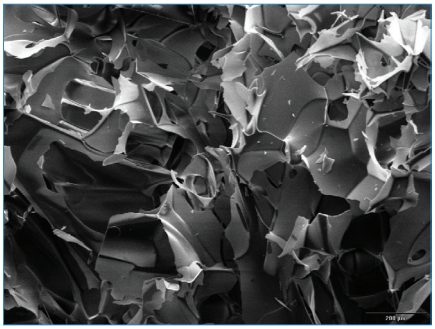


Figure 3: *Using controlled nucleation at -3°C ("CL@-3°C")*

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Implementing ControLyo Technology in a Commercial Environment

As described above, there are many different approaches to control ice nucleation in a laboratory-scale freezer, however many of these are not appropriate for commercial purposes. For example, patient safety could be compromised by biological contaminants and toxic issues with the addition of nucleating agents. However, it is relatively easy to implement ControLyo technology into a commercial dryer that is capable of steam-in-place (SIP) and has available ports to evacuate the chamber in the required time. Only minimal changes need to be made to the system and therefore, there is a nominal impact on existing validation and protocols.

One of the benefits of ControLyo implementation is a new control interface which is simple to use while still complying to FDA's 21 CFR Part 11 regulations on electronic record keeping. Its software also enables any type of cycle (even those that do not require CIN) to be run without any changes to the internal and external equipment making this technique a practical solution to maximizing usage and improving production output.

SP Scientific's LyoStar 3 freeze dryer with ControLyo technology was one of the first commercially available freeze dryers with the capability to control nucleation. The success of ControLyo over the years has made it now possible to retrofit freeze dryers with this technology regardless of manufacturer. The retrofit can repurpose existing production equipment and expand the capabilities of the freeze dryer although other cycles that do not require ControLyo can still be run on the existing equipment. The retrofit is time efficient as it is generally shorter than the time taken for the manufacturing cycle for a new freeze dryer.



S20 LyoConstellation Freeze Dryer

SP Scientific also provides a freeze dryer refurbishing service to extend the life of existing equipment by updating, repairing, refurbishing, retrofitting previously owned lyophilization systems including retrofitting and upgrading controls system. This LyoRenewal capability enables each installation to be customized to the company's standard operating and control procedures and other site requirements or corporate policies.

LINE OF SIGHT ENABLED

Scaling Up Lyophilization from R&D to Commercial Product Manufacturing

The critical parameters that lead to efficient production of a quality product are the same, whether it is in early-stage development, clinical stages, pilot batches or commercial manufacturing. Although these factors are the same at each lyophilization step, transferring from one stage to another often requires repeated optimization of these parameters due to the differences between equipment at each stage. Understanding the limitations of the commercial freeze dryer before embarking on scaling up the drug development process can reduce optimization time for this process.

ControLyos technology is based on pressure and therefore, each vial, regardless of shelf location or tray position experiences very similar conditions at the same time. When scaling up from lab to commercial production, the ControLyo nucleation process remains the same; pressurizing to 28.5 PSIG and depressurizing to 2 PSIG in less than 2 seconds. Successful nucleation has also been demonstrated with ControLyo at pressures as low as 17.5 PISG to 2 PSIG in a 96 ft^2 chamber. The only requirement of the commercial freeze dryer for ControLyo technology is that there is an appropriate port or combination of ports to achieve the speed needed to achieve ice nucleation.

ControLyo is part of a suite of tools (technologies and equipment) created by SP Scientific called Line of Sight® (LoS) that can be employed at each stage of development and production for increased control, efficiency, quality and consistency in the lyophilization process. This suite of technologies and PAT tools (SMART™, LyoFlux® TDLAS, Tempris® and ControLyo®) are built into small-scale freeze dryers (LyoCapsule™, LyoStar™ 3) through to large scale commercial dryers (LyoConstellation™) which ensures the use of technology and methodology from one equipment can be reproduced and compared directly to another. This approach provides greater confidence in the methodology and meaningful comparison of the results at each stage.

Process Changes and Meeting Regulatory Compliance

To assure quality product performance in drug manufacturing, the US Federal Drugs Administration (FDA) strongly recommend a Quality by Design (QbD) approach. This includes better product knowledge and greater control of the lyophilization process. As with any changes to a process, it needs to be determined that product quality (e.g. strength, purity, and potency) and long-term stability using ControLyo is comparable or better than the current process. The main difference between ControLyo and other freeze-drying techniques is the use of pressurization and depressurization, as described earlier, that leads to simultaneous controlled ice nucleation. It is, therefore, not expected that there would be a need for concern. Still, the FDA require a series of evaluation experiments to determine any effect of ControLyo on product attributes. However, if there are changes that fall outside the established design space, implementing ControLyo into the lyophilization process will require the end-user to submit a supplement (21 314.70 (e) or 21 601.12 (for a biological drug)) to the approved New Drug Application (NDA) or Biological License Application (BLA).

Concluding Remarks

One of the keys to successful lyophilization is the ability to control ice nucleation so that each vial freezes at the same time and produces a high-quality product with less variability and greater efficiency. Although there are several methods to achieve controlled nucleation, discussed in this article, SP Scientific's ControLyo technology using a series of pressurization and depressurization steps has been proven to achieve all these goals. ControLyo technology increases the homogeneity of vials and between batches, decreases cycle time, improves cake morphology and reduces vial breakage.

Implementation of ControLyo is fast and efficient, requiring minimal downtime and having little impact on existing validation which also reduces the effect on resubmitting supplement applications for FDA approval. It is also possible to retrofit ControLyo onto existing equipment regardless of manufacturer making ControLyo a decidedly versatile technology. ControLyo's advantages are not only within a small-scale freeze dryer, but ControLyo can be reproduced and compared directly to other larger freeze dryers during product scale-up with minimal effort through SP Scientific's LoS suite of technologies. This enables a product to move through different stages of development to commercialization more efficiently.

Overall, ControLyo streamlines the development process and establishes consistent product quality for lower long-term economic burden and support of best patient outcomes.

References

[1] Wang B.S. and Pikal M.J. (2012) Stabilization of Lyophilized Pharmaceuticals by Process Optimization: Challenges and Opportunities. Am. Pharma. Review https://www.americanpharmaceuticalreview.com/Featured-Articles/122325-Stabilization-of-Lyophilized-Pharmaceuticals-by-Process-Optimization-Challenges-and-Opportunities/

[2] Searles J.A., Carpenter J.F. and Randolph T.W. (2001) The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperature-controlled shelf. J. Pharm. Sci. https://doi.org/10.1002/jps.1039

[3] Mudhivarthi V.K. (2015) Controlled ice nucleation in freeze-drying: freezing heterogeneity and protein stability. SP Scientific Lyolearn Webinar.