The Importance of Controlling Nucleation Temperature during the Freeze Step. Introduction of *ControLyo*™ Nucleation On-Demand Technology on the New FTS/SP Scientific™ LyoStar™3 Freeze Dryer

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Introduction

The importance of nucleation temperature in freeze drying has been known for some time. A number of earlier techniques to control nucleation, while demonstrating the potential for process improvement, were not easily transferrable to development or production freeze dryers, from a commercial standpoint. These include the "Ice Fog" technique described by Rambhatla et al.[1] and ultrasound, published by Hottot et al.[2]. Recently, a novel technology for controlling nucleation was described by Praxair Inc. This method, called *ControLyo™* Nucleation On-Demand Technology, has been shown to significantly improve both product and process in many experimental systems.[3] *ControLyo* technology is now commercially available on the new FTS/SP Scientific, Lyostar 3 development freeze dryer. This technology has been fully integrated and can easily be practiced even by relatively inexperienced users. Additionally, existing commercial freeze dryers can be modified or upgraded to accommodate the technology, if they have ASME rated pressure chambers (e.g. SIP systems) and sufficiently sized orifices for depressurization. This paper reviews ice nucleation, describes the ControLyo technology and shows data for controlled nucleation on the Lyostar 3. Additionally, application of the technology in a number of freeze drying environments (i.e., size of dryer, load, formulation, container size, etc.) is presented.

Review of Nucleation

"Control and characterization of the degree of super-cooling can provide a solution to what is perhaps the biggest freeze drying scale-up problem"[4]

This quote from a paper by Pikal et al, sets the stage for the discussion below. A primary goal of cycle development and scale-up is to establish optimum cycle parameters while maintaining product quality. A well optimized process can reduce the cycle time (and therefore cost) in freeze drying. In our experience, this is mainly done by focusing on reducing the time of primary drying, since this is usually the longest part of the cycle. Optimization of the freezing step is rarely a focus. However, to a large extent, the temperature at which freezing takes place, and the degree of super-cooling that occurs determine how much optimization can be done to reduce the length of primary drying. Part of the reason that the freezing step has been largely ignored is that development scientists have not had access to tools and technology to impact this step. Other than annealing, the freezing step has been an unexploited opportunity to improve the freeze drying process.[5]

To better understand the benefits of *ControLyo* technology in the context of optimizing the freezing step, it is helpful to understand the basics of nucleation and super-cooling. These physical behaviors are summarized below:

1. Nucleation temperature is the temperature at which freezing or ice crystal formation begins to take place.
2. Nucleation rarely occurs at the equilibrium, or thermodynamic freezing point of the solution. In a lab or development freeze dryer, it is common for nucleation to occur 10-20°C below the thermodynamic freezing point. In a commercial freeze dryer (Class 100 Clean Environment), nucleation tends to occur 20-30°C below the thermodynamic freezing point, and may even exceed that level. In this clean environment, there are fewer particulates that can serve as nucleation points during the freezing step.
3. The degree of super-cooling can have a dramatic impact on the drying behavior.
   a. High degree of super-cooling = smaller ice crystals = smaller pores during sublimation. This increases resistance to mass transfer and increases primary drying times.
   b. Low degree of super-cooling = larger ice crystals = larger pores during drying. This reduces resistance to mass transfer and decreases primary drying times.
4. This effect can be significant. Previous studies have demonstrated that for every 1°C increase in the nucleation temperature, primary drying time can be reduced by as much as 3-4%.[6]

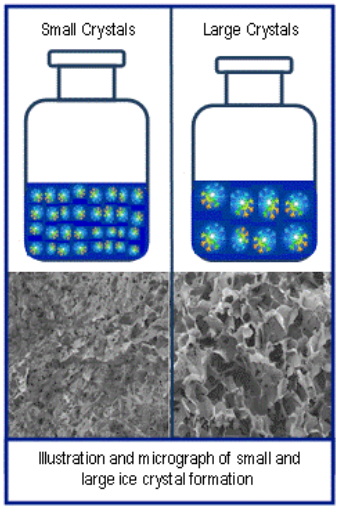

Figure 1.

Figure 2 shows the temperature of a vial filled with water during freezing. As the vial is cooled, it passes through the Thermodynamic Freezing Point = Tf, which for water is 0°C, and below which nucleation occurs. Nucleation is an exothermic event, and this is observed in figure 2 by the rapid temperature spike that approaches the thermodynamic freezing point. The nucleated contents of the vial then cool, following a temperature history dictated by the cooling ramp of the shelf. The point at which nucleation begins is called the Temperature of the Onset of Nucleation = Tn. The difference between Tf and Tn is the degree of super-cooling of the sample.

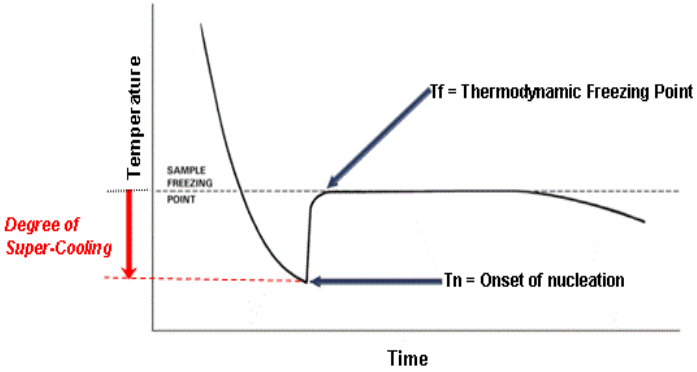

Figure 2. *Typical Freezing Curve*

Uncontrolled Nucleation Results

Figure 3 shows the results of uncontrolled nucleation during the freezing step in a cycle run on a LyoStar 3 (**Figure 4**). This is typical behavior in a lab freeze dryer. A tray was loaded with 10ml vials of 3% sucrose with a fill volume of 2.0 ml. Self-adhesive thermocouples were adhered to the outside of 16 vials, including edge and interior vials. The outside placement prevents unintended nucleation resulting from the thermocouple itself.

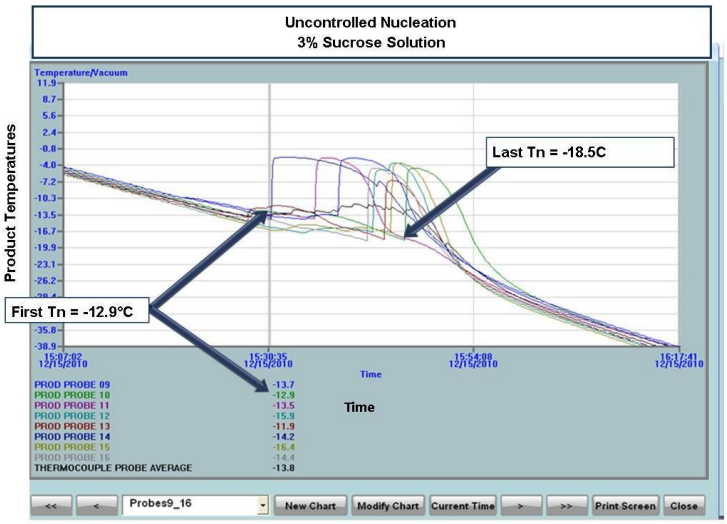

Figure 3. *Graph of uncontrolled nucleation*

Figure 4. LyoStar 3 with *ControLyo™* Nucleation On-Demand Technology

The graph shows the thermocouple data from 8 vials. During a 0.5°C/min ramp from -4°C to -45°C, the first nucleation event occurs at -12.9°C. Random nucleation of other vials occurs over an approximately 15 minute period, with the final nucleation event occurring at -18.5°C, approximately 18°C below the thermodynamic freezing point of the sucrose solution. Using the data supplied by Searles, Carpenter & Randolph (6), the drying time of the last vial to nucleate could be almost 20% longer than the first vial, and 45% longer than a vial made to freeze close to its thermodynamic freezing point by controlled nucleation!

Longer cycle times are not the only consequence of uncontrolled nucleation during freeze drying. Other adverse effects which have been observed, or are known industry concerns are shown in **Table 1**.

Adverse Effects of Uncontrolled Nucleation	
Cycle Development	Complex formulations and conservative cycles help mitigate nucleation problems adding costs and long cycles
	Unpredictable process scale-up and transfer due to performance variations due to freeze dryers and environments
Manufacturing Cost & Capacity	Colder nucleation creates smaller ice crystals – slows drying rate, cycles take longer, cost more
	Longer cycles require more facility capacity and higher operating costs
Product Yield	Uncontrolled nucleation can create an ice structure that damage API's
	Improper freezing will exacerbate vial cracking and breaking
	Risk of product loss increases with longer cycles because of dwell time in freeze dryer
Product Quality	Vial-to-vial uniformity during freezing (and consequently freeze drying) is impossible
	Poor homogeneity or cosmetic elegance for certain products
	Lack of process control is not aligned with FDA, QbD initiative

Table 1. *Adverse effect of uncontrolled nucleation* [3]

Controlled Nucleation Results with Praxair *ControLyo*™ Nucleation On-Demand Technology

The *ControLyo* technology is elegantly simple and easy to use on the Lyostar 3. A typical controlled nucleation run is described below:

a. Load vials onto shelves pre-cooled to 4°C.
b. After loading, reduce the temperature to -4°C (or whatever your desired nucleation temperature is) using a 0.5°C/min shelf ramp rate.
c. After reaching -4°C, pressurize the system with an inert gas (nitrogen or argon) to approximately 25psig (1.7 bar).
d. Equilibrate at -4°C for as long as necessary to achieve vial-to-vial temperature homogeneity (e.g. 30-45 min.).
e. After equilibration, rapidly depressurize the system to approximately 1 psig (0.069 bar) to induce nucleation.
f. Hold at -4°C for 15-20 minutes to promote ice crystal growth and limit formation of secondary nucleation centers at colder temperatures.
g. Ramp down to -45°C at 0.5°C/min to complete the freezing step.

The primary drying phase is now ready to begin. All *ControLyo* technology steps are easily programmed into the cycle recipe in the Lyos Software on the Lyostar 3, as shown in **Figure 5**.

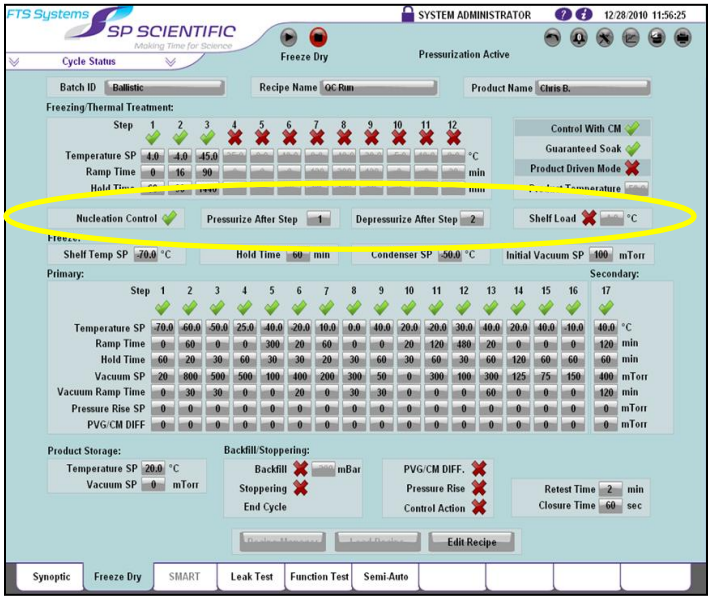

Figure 5. *LyoStar 3 Lyos Software with ControLyo™ Nucleation On-Demand Technology*

Please note that *ControLyo* technology cannot force nucleation at temperatures above the thermodynamic freezing point of the solution. It also cannot prevent random nucleation of super-cooled vials if one targets a nucleation temperature significantly below the thermodynamic freezing point.

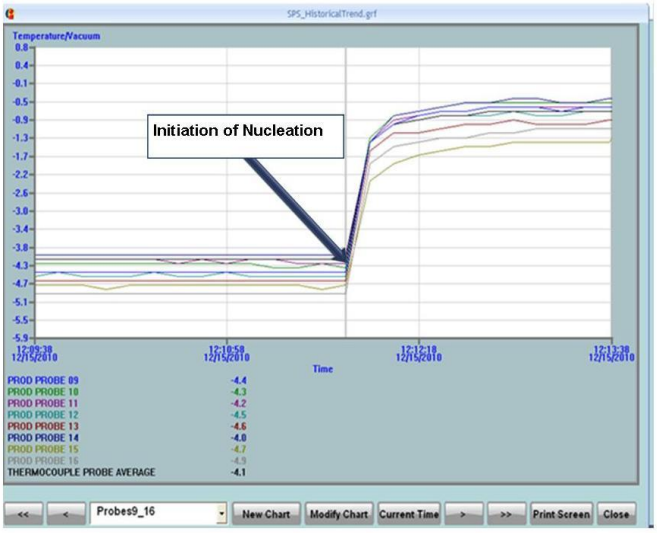

Figure 6. *Graph of controlled nucleation*

Figure 6 shows controlled nucleation results in the Lyostar 3. The same experimental design as described above (vials, contents, fill volume and thermocouple placement) was used. The vials were cooled to -4°C and equilibrated for 45 min, while the system was pressurized with argon at approximately 25 psig. The system was then rapidly depressurized using Praxair's *ControLyo* technology. As can be seen from the graph, all vials nucleated simultaneously. The run was then terminated and the vials examined visually to verify all vials had nucleated. A dramatic video, filmed inside of a freeze dryer by Praxair during controlled nucleation, can be seen on the SP Scientific web site at www.spscientific.com. This video shows uncontrolled nucleation in fast forward over a period of an hour and controlled nucleation in real time.

Figure 7. *Photo of uncontrolled nucleation in a freeze dryer.*[7]

Figure 8. *Photo of controlled nucleation in a freeze dryer.*[7]

Praxair's *ControLyo*™ Nucleation On-Demand Technology Demonstrations	
Implementation	Praxair: 0.6, 1 & 5 m^2 Freeze Dryers Lyophilization Services of New England: 1 & 5 m^2
Temperature	nucleation controlled at temperatures as warm as -1°C
Batch Size	3,150 vials (three full shelves in 5 m^2 freeze dryer)
Formulations	mannitol, HES, PEG, PVP, dextran, glycine, sorbitol, sucrose, trehalose, cyclodextrins, citrate and Tris buffers
Model Proteins	bovine serum albumin, bovine IgG, lactate dehydrogenase, horseradish peroxidase, glucose-6-phosphate dehydrogenase
Products	6 drug formulations provided by outside companies
Containers	2 mL vials to 100 mL bottles to bulk trays
Fill Volumes	wide range

Table 2. Demonstrations of *ControLyo* technology.[3]

While the experimental data clearly illustrates the efficacy of the Praxair's *ControLyo* technology in the Lyostar 3, it is based on a non-commercial model system (3% sucrose solutions, 10ml vials, no API, at -4°C). In order to prove the applicability of the technology to a wide range of commercial environments, Praxair has performed a large number of runs using different "real world" freeze dryers, batch sizes, formulations, vial sizes, etc. The *ControLyo* technology worked successfully in all cases, demonstrating its wide ranging capabilities in freeze drying.[3]

Experimental Demonstration of the Benefits of Controlled Nucleation

Of even greater significance are the experimental results obtained by the Praxair Team and in conjunction with their collaborations with University of Connecticut, Baxter BioPharma Solutions and Lyophilization Services of New England. Their results clearly show a number of improvements to products and/or processes that can be obtained by controlling nucleation. These results are summarized in **Table 3** and may be viewed in detail in the presentation given at the 2010 CPPR Meeting in Garmisch, Germany by Dr. Robert Sever of Praxair.[7]

Benefits of *ControLyo*™ Nucleation On-Demand Technology	
Results	**Benefits**
Reduction in dry layer resistance of 52%, 27% and 58% in three (3) different formulations using controlled vs. uncontrolled nucleation	Increase in pore size and reduction in dry layer resistance
Demonstrated 41% faster primary drying with controlled nucleation of 5wt% mannitol	Faster primary drying
Incidence of aggregation with Lactate Dehydrogenase formulation reduced from 16 of 24 cases (67%) to 6 of 24 cases (25%)	Reduced freezing stress on biologicals
Improved cake structure and cosmetic appearance with Vancomycin	Improved cake appearance
Human Growth Hormone (hGH) aggregation reduced in controlled vs. uncontrolled nucleation	Reduced protein aggregation

Table 3. Benefits of *ControLyo*™ Nucleation On-Demand Technology.[7]

Future Development Opportunities

By implementing Praxair's *ControLyo* technology on the FTS Lyostar 3, the development scientist now has two extremely powerful process development, cycle optimization and scale-up technologies on one freeze dryer: SMART/MTM and *ControLyo*™ Nucleation On-Demand technology. SMART's value as a cycle development and PAT tool has been widely described.[8,9] and SMART has become the technology of choice by most major Biopharma companies worldwide. The combination now gives researchers capabilities never before available on a freeze dryer. For example, nucleation temperature can be controlled with *ControLyo* technology and the cycle can be automatically optimized using SMART. The ability of SMART to measure and/or calculate a number of critical product and process parameters (This includes: Rp = Product Resistance, Tp = Product Temperature at Ice Surface Interface, Tb = Product Temperature at the Bottom of the Vial, Lice = Ice Thickness and dm/dt = Sublimation Rate) allows the researcher to study the impact of nucleation temperature on these parameters. The combination of SMART and ControLyo Technology should significantly enhance cycle optimization and scale-up in freeze drying.

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