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# Short review on Controlled Nucleation

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**Abstract**

Freeze-drying (also known as lyophilization or cryodesiccation) is a dehydration process mainly used to preserve for perishable material and this method make the material more convenient for transport. Freeze-drying works by freezing the material and then reducing the surrounding pressure and giving sufficient heat to allow the frozen water into the material to sublime directly from the solid phase to gas phase. Sublimation is the transition of a substance from the solid to the vapor state, without first passing through an intermediate liquid phase. A method to achieve controlled ice nucleation during the freeze-drying process using an ice fog, vial treatment, ultrasound, additives and electro freezing technique was demonstrated in an earlier report. And by the potential of a high electric field was utilized to induce ice nucleus formation in aqueous solutions. Using this technique it was possible to reduce the primary drying time during lyophilization. [1]

Many technical issues surrounding the freeze-drying process have been addressed over the past several decades. Better understanding of critical formulation characteristics and cycle conditions. There are two types of technique for nucleation 1) Uncontrolled Nucleation, 2) Controlled Nucleation. The objectives of the present study simply introduce controlled Nucleation is the rapid ice nucleation in freeze drying technique. [2]

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## INTRODUCTION:

Lyophilization or freeze-drying is often used to stabilize various pharmaceutical products, including virus vaccines, protein and peptide formulations, liposome and small-chemical drugs susceptible to physical and chemical degradation when stored as a ready-to-use solution. [9, 10, 11, 12] Freeze-drying is carried out in three steps: freezing, primary drying, and secondary drying. During the freezing step, most

of the water is converted into ice at temperatures in the vicinity of −40°C. Then, the system is evacuated using vacuum pumps, and the shelf temperature is raised to facilitate ice sublimation during primary drying. In the secondary drying stage, the shelf temperature is further raised to efficiently remove unfrozen water by desorption to provide a low residual water content, typically less than 1%. Reproducibility of the freezing process is the subject of this research, in particular control of the degree of super-cooling. [8]

An important objective of the freezing step is to produce a homogeneous batch, which is challenging because of the random nature of nucleation. The ice nucleation temperature, Tn, is quite variable even in a well-controlled process.

The degree of supercooling, defined as the difference between the equilibrium freezing point and the temperature at which ice crystals first form in the sample, reflects random nucleation and also depends on the solution properties and process conditions.[7]

The ice nucleation process is spontaneous and stochastic in nature and depends on the solution properties, process conditions, the surface characteristics of the container, and the presence of particulate matter that may serve as heterogeneous nucleation sites. During primary drying, water vapor flows through the void channels previously filled with ice. A higher degree of super-cooling results in more ice nuclei, meaning smaller ice crystals since the total amount of ice is fixed. Thus, greater super-cooling results in smaller pore size, greater resistance to vapor flow, and longer primary drying time.

Smaller pore size not only results in higher resistance to flow of water vapor during primary drying but also means greater surface area and hence faster desorption during secondary drying. [8]

**Uncontrolled Nucleation & Controlled Nucleation:**

- **Adverse affect of Uncontrolled Nucleation:**

**Manufacturing Cost and Capacity:** The significant subcooling that occurs in most vials before nucleation leads to formation of smaller ice crystals. It is generally recognized that smaller ice crystals reduce the primary drying rate because mass transfer is limited through the small pores they leave behind as they sublimate. It is estimated that for every degree increase in nucleation temperature, there is a 1–3% decrease in drying time. As a result of subcooling, the primary drying step must be run excessively long to accommodate the slowest-drying vials [3,7]

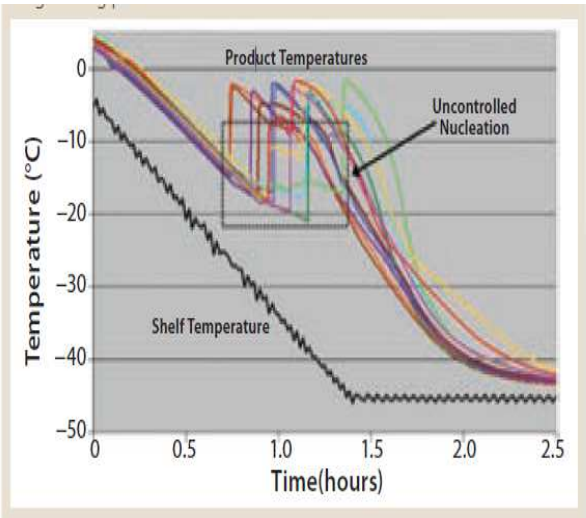



**Figure 1:** Uncontrolled Nucleation

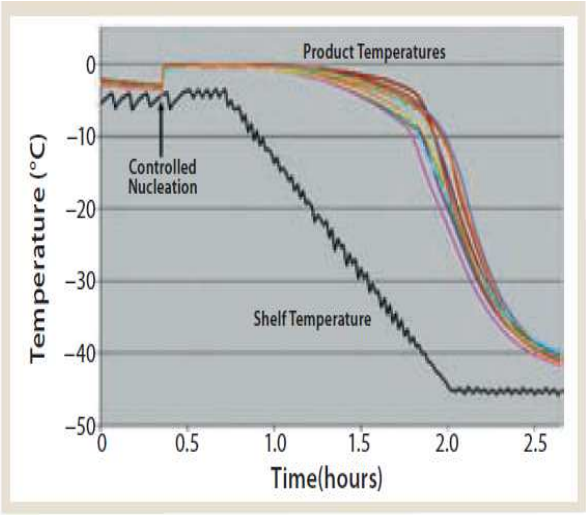
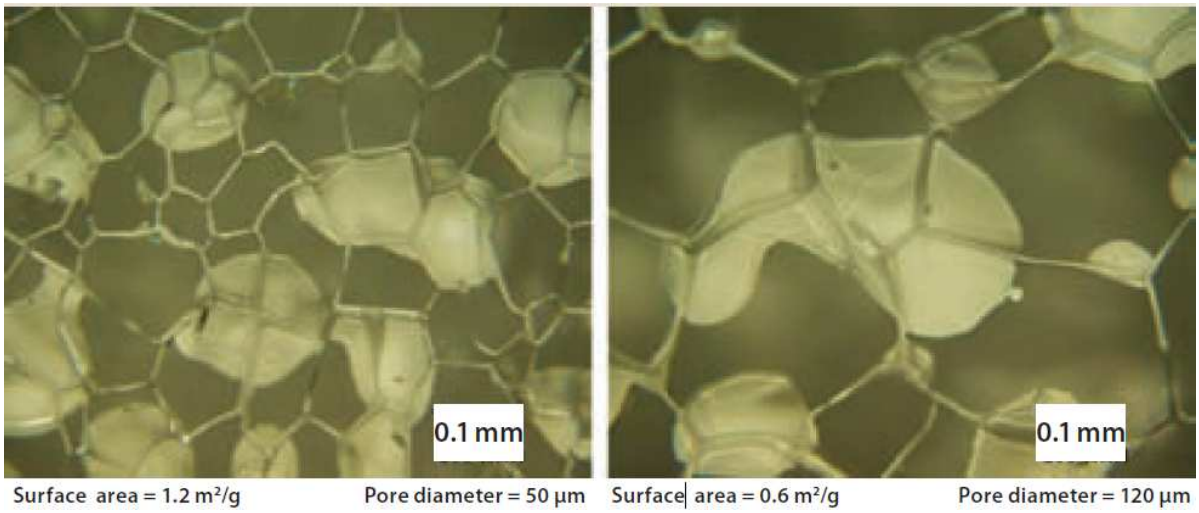



**Figure 2:** Controlled Nucleation

Figure 1 shows an example of this stochastic nucleation behavior and Figure 2 shows an example of this controlled nucleation. [2]

**Product Yield:** Although lyophilization is considered a relatively gentle preservation method, the inherent freezing stresses can negatively affect product yield, particularly for sensitive biologics.



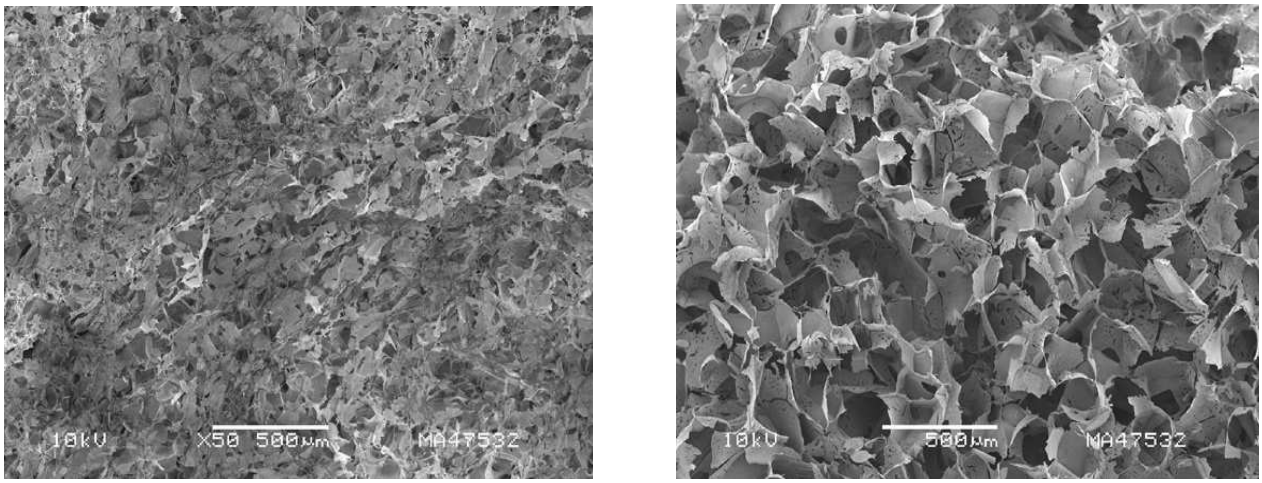




**Figure 2:** Shows an example of this stochastic (Uncontrolled) nucleation behavior and controlled nucleation. [2-4]

**Product Quality:** Uncontrolled nucleation introduces significant heterogeneity because nucleation behavior strongly influences product microstructure, rate of drying, crystallization of excipients, and the local preservation environment of an active ingredient. [2-3]

**Cake Microstructure:** The microstructure of cakes lyophilized with conventional stochastic nucleation and control nucleation method have been examined with scanning electron microscopy (SEM), polarized light microscopy, and gas adsorption. All three methods verify the expectation that controlled nucleation at warmer temperatures produces substantially larger pores than stochastic nucleation does. Figure. 2 compares pore dimensions. [2-4]

**Control Nucleation:**

Stochastic nucleation is a well-recognized gap in lyophilization process control. Significant work has been gone toward elucidating the problem and

exploring various control methods and their potential for commercial application.

1. **Ice Fog:** Ice crystals can themselves act as nucleating agents. In the "ice fog" method originally described by Rowe in 1990 [4] and demonstrated at laboratory scale by Rambhatla et al.[5] a humid freeze-dryer is filled with a cold gas to produce a vapor suspension of small ice particles. The degree of super-cooling is defined as the difference between the equilibrium freezing point and the ice nucleation temperature (Tn), which is the temperature at which ice crystals are first formed in the solution. The nucleation temperature governs the number of ice nuclei formed during the freezing step, which in turn affects the product resistance and temperature during the drying stage and, therefore, the drying time. The ice nucleation process is spontaneous and stochastic in nature and depends on the solution properties, process conditions, the surface characteristics of the container, and the presence of particulate matter that may serve as heterogeneous nucleation sites. During primary drying, water vapor flows through the void channels previously filled with ice. A higher degree of super-cooling results in more ice nuclei, meaning smaller ice crystals since the total amount of ice is fixed. [8]

Annealing is commonly used during the freezing step to overcome the ice nucleation heterogeneity. Once ice nucleates in the samples, and the system is mostly frozen, usually at about −40°C, the shelf temperature is raised to a temperature above the glass transition temperature, Tg, of the formulation but below the onset of ice melt. Due to the enhanced molecular mobility, larger ice crystals are formed at the expense of smaller ones, a phenomenon referred to as Ostwald ripening (see figure 3). Thus, annealing can eliminate, or at least minimize, the differences in pore size and drying rate caused by different degrees of super-cooling. [8]

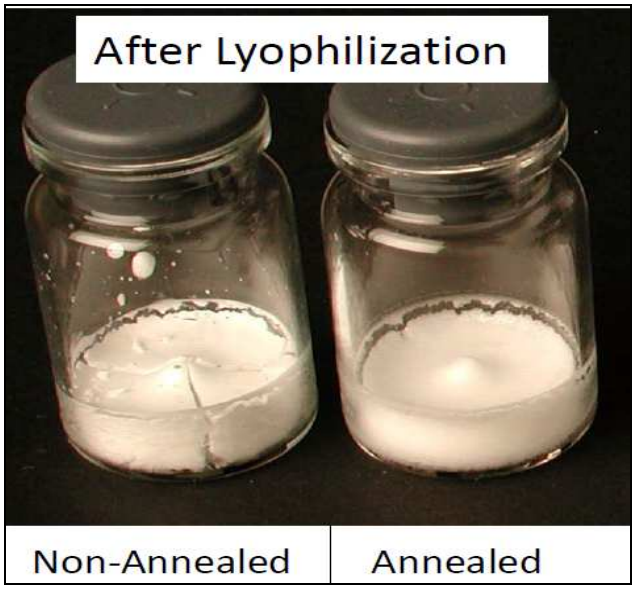



**Figure 3:** Shows an example of non-annealed and annealed product.

In this technique, the product temperature is reduced to the desired ice nucleation temperature, nitrogen gas is passed through copper coils immersed in liquid nitrogen and then introduced into the product chamber, thereby producing a dense ice fog. The ice fog is forced into the vials, seeding the crystallization of ice. In the previous report, a tube with uniformly punched holes was clamped above the shelf to achieve a uniform distribution of ice fog. In the previous report, a tube with uniformly punched holes was clamped above the shelf to achieve a uniform distribution of ice fog . However, in this variation of the technique, the chamber was maintained at atmospheric pressure, and it was found that even with only using one shelf of vials, the time required for nucleation was about 5 min, which resulted in some Ostwald ripening or annealing in some of the vials that nucleated earlier than the other vials. [4]

- **Reduced pressure ice fog technique:** The combination of ice fog with reduced pressure is referred to as the Reduced Pressure Ice Fog Technique. When the desired ice nucleation temperature was reached (−10°C), the vacuum pump was turned on to reach the optimized chamber pressure (48–50 Torr.). The chamber was then

isolated by closing the valve connecting the chamber and the condenser. To execute the ice fog procedure, nitrogen gas was passed through copper coils immersed in liquid nitrogen and introduced into the chamber through one of the inlet ports available on the top of the chamber. As the cold nitrogen gas enters the chamber, the chamber pressure begins to increase, ice forms, and the ice fog is forced into the vials. As the chamber pressure approaches atmospheric pressure, the isolation valve to the condenser was quickly opened, and the ice fog was pumped into the condenser. The ice nucleation temperature of −10°C was selected as it represents the maximum super-cooling that can routinely be achieved in a laboratory dryer under full-load conditions. Using a much lower nucleation temperature is difficult due to higher level of particulate matter in the laboratory (i.e., the vials nucleate even before the ice fog technique is executed).

This method faces challenges in controlling the nucleation of all vials simultaneously at a desired time and temperature for commercial scale freeze-dryers. Uniform distribution of the ice suspension may be difficult, and ice particle transport rates may be different for vials in different locations. Successful commercial-scale implementation of the ice-fog method has yet to be reported. [2,5]

Using a partial vacuum in the chamber during introduction of the cold nitrogen, the ice fog technique has been improved to allow more rapid and more uniform nucleation. The reduced pressure technique should also allow easier scale-up of the ice fog nucleation technique to production scale dryers. [8]

2. **Vial pretreatment** by scoring, scratching, or roughening has also been used to lower the degree of subcooling required for nucleation. This works by producing surface defects or glass nanoparticles that can catalyze nucleus formation. As with other additives, glass nanoparticles are generally undesirable for pharmaceutical products. Vial pretreatment imparts no control over the time and temperature when individual vials nucleate and freeze, but instead only increases their average nucleation temperature overall.

3. **Ultrasound:** The well-established ability of ultrasonic vibration to induce nucleation in subcooled solutions has been applied to lyophilization applications at small scales [6]. It is generally believed that disturbances caused by the rapid growth and collapse of gas bubbles under transient cavitation trigger nucleation. The major challenges of implementing this method in a commercial-scale freeze-dryer — with sufficient uniformity and without compromising equipment cleanability — have not yet been met.

4. **Additives:** In general, all additives/contaminants have the potential to serve as nucleating agents. The most commonly investigated agents include silver iodide, Pseudomonas syringae bacteria, and adventitious environmental particulates. The use of additives is not typically acceptable or desirable for lyophilization of FDA-regulated and approved pharmaceutical products. Additives provide insufficient control over time and temperature when individual product vials nucleate and freeze; they serve only to increase their average nucleation temperature.

5. **An electrofreezing method** has also been used to induce nucleation in subcooled solutions [13]. This is generally accomplished by delivering relatively high electric fields (~0.01 V/nm) either continuously or pulsed between narrowly spaced electrodes immersed in the solution to be freeze-dried. The need for individual electrodes in each vial makes this method truly impractical for use in commercial pharmaceutical manufacturing. Also,

electrofreezing cannot be applied directly to formulations that contain ionic molecules (e.g., NaCl).

**Conclusion:** The controlled ice nucleation technique resulted in rapid ice nucleation at the desired temperature under widely different process and formulation conditions. Thereby improving the uniformity of drying as well as eliminating what is perhaps the most important scale-up problem.

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