Application Note

LyoCoN – Controlled Nucleation

Content

Abstract

Controlled nucleation is a powerful tool for improving the reproducibility and structural uniformity of freeze-dried products. Unlike spontaneous, heterogeneous ice nucleation, LyoCoN actively triggers nucleation simultaneously across all vials in a batch. This produces a well-defined ice crystal structure, improves pore homogeneity, and enables tighter control of the sublimation phase.

Successful implementation depends on proper equipment configuration and careful selection of process parameters. Both insufficient and excessive supercooling can compromise nucleation efficiency and introduce unwanted variability.

Introduction

Freeze-drying (lyophilization) is widely used to enhance the long-term stability of pharmaceutical and biotechnological products. The process consists of three main stages: freezing, primary drying, and secondary drying.

The freezing step is critical because it defines the microstructure of the product. The structure of the frozen matrix directly determines the pore structure of the dried cake and therefore strongly influences drying performance during primary drying.

Most aqueous formulations form crystalline ice structures with clearly defined crystal boundaries. However, in standard freeze-drying processes, ice nucleation typically occurs randomly and without control. As a result, nucleation temperatures vary from vial to vial, leading to differences in ice crystal size and distribution within a batch. Batch-to-batch variability can be significant.

This structural variability can negatively affect process consistency, drying time, and ultimately product quality.

By controlling the onset of nucleation and triggering it simultaneously across all vials, manufacturers can create a uniform ice crystal structure. This improves batch consistency and enhances overall process robustness.

Fundamentals

Nucleation and Ice Crystal Formation

Freezing rarely begins at the equilibrium freezing temperature. Instead, the liquid phase undergoes supercooling as it departs from thermodynamic equilibrium. Supercooling of up to 20 K is possible.

Once nucleation is triggered, ice crystal formation begins immediately. Water molecules organize into a solid crystalline lattice, releasing latent heat of crystallization. This heat release causes the product temperature to rise rapidly toward the equilibrium freezing temperature. The nucleation temperature has a direct impact on ice crystal morphology:

High supercooling: many small ice crystals
Low supercooling: fewer, larger ice crystals

Ice formation is complete once all free water in the product has transitioned to the solid phase. Because nucleation temperature determines ice structure, it ultimately governs pore structure and drying resistance in the subsequent primary drying phase.

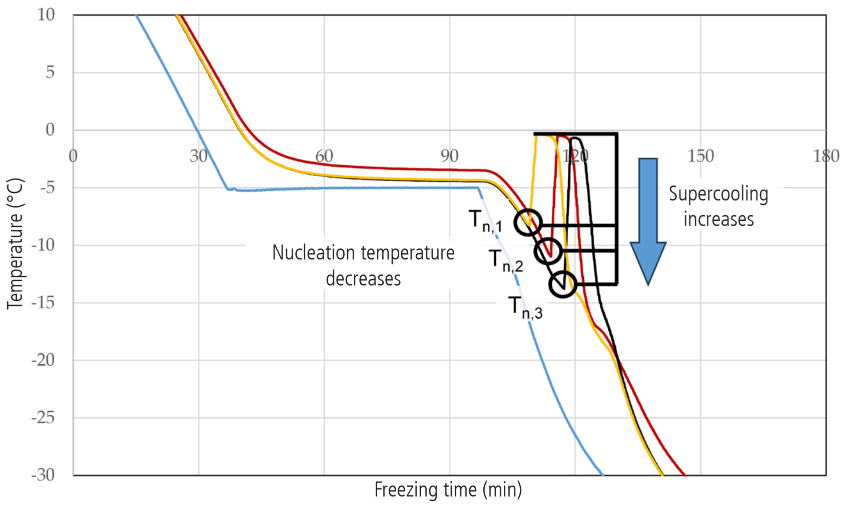


Figure 1: Distribution of nucleation temperatures during uncontrolled nucleation and its impact on supercooling.

Implementation

To enable simultaneous and controlled nucleation, the freeze dryer is equipped with a LyoCoN container and an automated aeration valve.
LyoCoN can be integrated into pilot- and production-scale freeze dryers with dual-chamber systems. Systems can be configured at the factory or retrofitted later. The technology is available from compact pilot units such as the Epsilon 2-6D LSCplus up to fully automated production-scale systems.

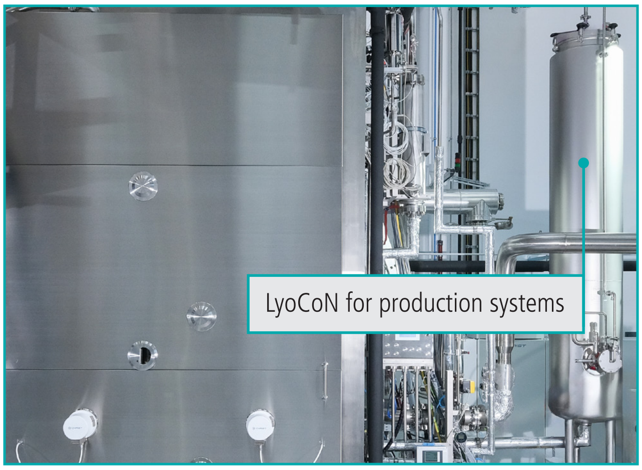


Figure 2: LyoCoN at different scales

Process Principle

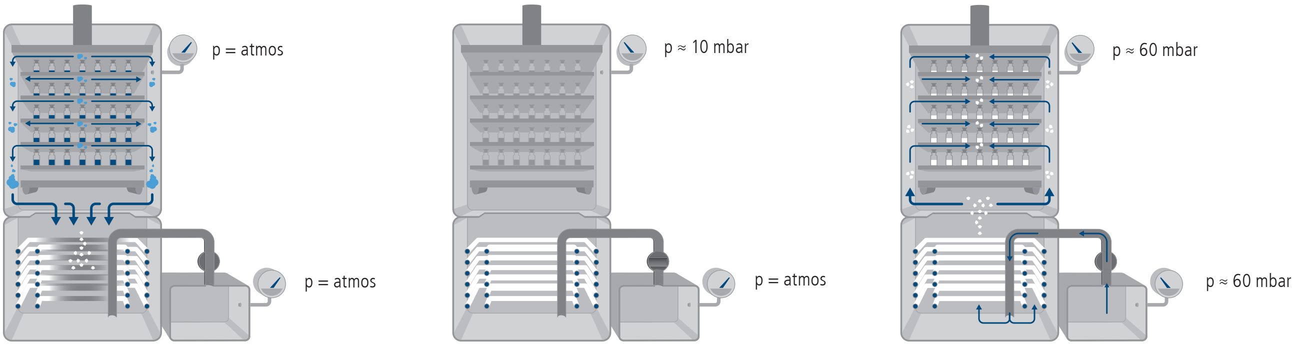
LyoCoN uses an ice fog technique to trigger nucleation.

In the first step, vials are cooled via the shelves to the target nucleation temperature. The temperature is then held to ensure uniform supercooling throughout the product. This hold step is essential because nucleation is initiated at the product surface. If the product is not sufficiently supercooled, nucleation will not occur.

During this preparation phase, ice crystals form on the cold condenser surface.

Next, a slight vacuum is applied inside the drying chamber while the external LyoCoN container remains at atmospheric pressure and is isolated by a valve. When the valve opens, pressure equalization occurs. The gas flow passes over the ice-covered condenser surface, entraining extremely fine ice crystal particles.

This ice fog enters the drying chamber and acts as a nucleation trigger inside the vials, initiating ice formation simultaneously across the batch.



Step 1: Preparation **Step 2:** Evacuation **Step 3:** Nucleation

Figure 3: LyoCoN process sequence

LyoCoN Parameters
The following parameters can be adjusted to match the nucleation process to a specific formulation:

- **LyoCoN Time [min]:** Hold time at the selected nucleation temperature to ensure uniform supercooling
- **LyoCoN Vacuum [mbar]:** Target chamber pressure during evacuation
- **LyoCoN max vacuum [mbar]:** Up to +10% of the set LyoCoN vacuum
- **LyoCoN min vacuum [mbar]:** Down to –10% of the set LyoCoN vacuum
- **LyoCoN stabilization time [s]:** Time during which chamber pressure must remain between the defined minimum and maximum limits

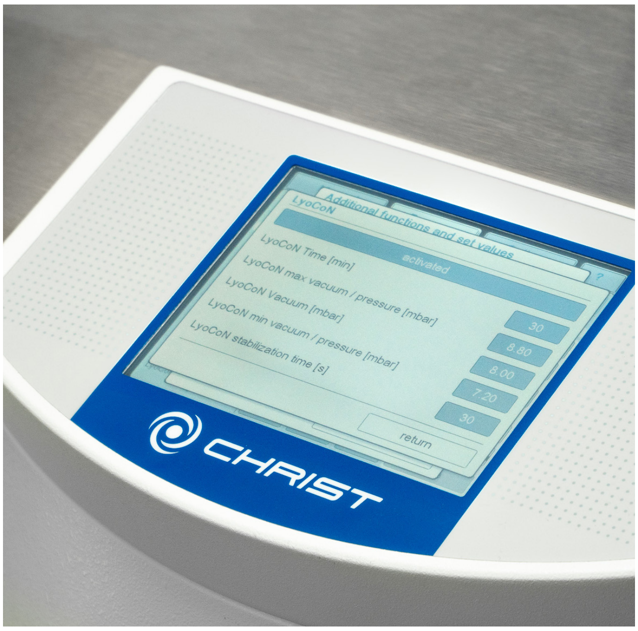


Figure 4: LyoCoN Control Interface

Practical Application
Once the freeze dryer is equipped with LyoCoN, the focus shifts to robust routine operation.

The most critical parameter is the nucleation temperature. It must be high enough to allow controlled, reproducible nucleation across the entire batch — but low enough to ensure sufficient supercooling.

Two boundaries must be respected:

- **Insufficient Supercooling**
  If the nucleation temperature is set too close to — or above — the equilibrium freezing temperature, the ice fog will not initiate nucleation. The product will continue cooling and eventually freeze via uncontrolled heterogeneous nucleation.
- **Excessive Supercooling**
  If the product is supercooled too strongly, spontaneous nucleation may occur before the LyoCoN trigger. In this case, freezing will not occur uniformly across all vials, leading to increased product variability.

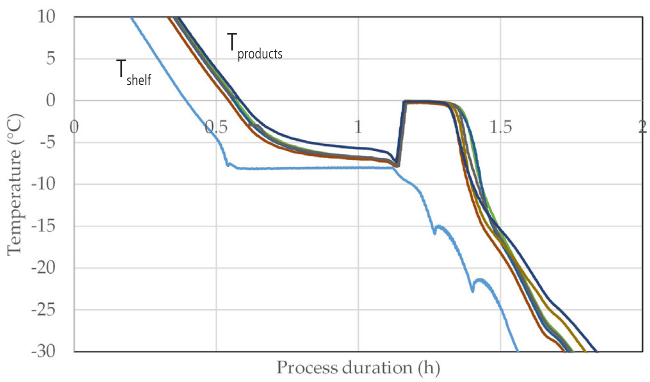


Figure 5: Distribution of nucleation temperatures during controlled nucleation and its impact on supercooling.

Video
Controlled nucleation

Model Study

A model study was conducted using sucrose solutions at concentrations between 1% and 20%, filled into 6R vials. Selected formulations were supplemented with sodium chloride (NaCl) at 0.1% or 0.9%.

The objective was to identify process conditions that enable simultaneous, controlled nucleation using LyoCoN and to assess the impact of salt content and solids concentration.

At a nucleation temperature of –1°C, controlled nucleation of the full batch could not be achieved. This temperature is too close to the equilibrium freezing temperature, and supercooling is insufficient to support nucleation.

At –4°C and –8°C, nearly all tested combinations resulted in successful controlled nucleation across the batch. The only exception occurred at a shelf temperature of –8°C combined with a chamber pressure of 10 mbar and a 20% sucrose formulation. Under these conditions, isolated vials nucleated prematurely and uncontrollably.

At –4°C, supercooling was sufficient to allow nucleation triggered by the ice fog while avoiding premature spontaneous nucleation.

At –8°C, certain formulations already exhibited excessive supercooling. This effect became more pronounced at –10°C, where premature uncontrolled nucleation occurred more frequently. At these temperatures, reproducible controlled nucleation cannot be ensured.

Within the experimental design evaluated, variations in salt concentration and solids content did not significantly affect nucleation behavior under the selected process parameters.

Sucrose		**1 %**	**5 %**	**10 %**	**15 %**		**20 %**		
Sodium chloride (NaCl)		**0**	**0**	**0**	**0**	**0.1 %**	**0**	**0.1 %**	**0.9 %**
T_{shelf}	$P_{chamber}$	-	-	-	-	-	-	-	-
–4 °C	2 mbar	●	●	●	●	●	●	●	●
	4 mbar	●	●	●	●	●	●	●	●
	7 mbar	●	●	●	●	●	●	●	●
	10 mbar	●	●	●	●	●	●	●	●
–8 °C	2 mbar	●	●	●	●	●	●	●	●
	4 mbar	●	●	●	●	●	●	●	●
	7 mbar	●	●	●	●	●	●	●	●
	10 mbar	●	●	●	●	●	○	●	●

● Successful controlled nucleation ○ Uncontrolled nucleation

Summary

Controlled nucleation is a highly effective strategy for improving freeze-drying reproducibility and product quality.

By precisely defining the onset of ice formation, variability in ice crystal structure is reduced. This leads to:

- Improved batch-to-batch consistency
- Greater process stability
- More uniform product structure
- Enhanced scalability

The approach aligns well with Quality by Design (QbD) principles by improving control over critical process parameters and product attributes.

LyoCoN applies an ice fog technique in which ice crystals formed on the condenser are introduced into the product via controlled pressure equalization. These crystals act as nucleation seeds and initiate freezing in a synchronized manner.

Successful implementation requires careful process development. Proper parameter selection — particularly nucleation temperature — is essential.

- Excessive supercooling may cause premature spontaneous nucleation.
- Insufficient supercooling may delay or incompletely trigger ice formation.

Both scenarios introduce structural variability and may compromise product quality.

The study presented here demonstrates that, for a wide range of formulations, a nucleation temperature of –4°C provides reproducible and uniform results.

By introducing controlled nucleation, manufacturers can significantly enhance the reliability and scalability of freeze-drying processes. LyoCoN therefore represents a valuable technology for achieving high standards of product stability and quality in pharmaceutical and biotechnological applications.

Checklist

1. **Preparation**
   - ☐ Verify that the system is equipped with LyoCoN

2. **Process Development**
   Characterize uncontrolled freezing behavior of the formulation
   - ☐ Perform freezing studies using gradually cooled shelves
   - ☐ Document nucleation temperature distribution and equilibrium freezing temperature

   Define nucleation conditions
   - ☐ Ensure sufficient supercooling (–4°C has shown strong performance)
   - ☐ Maintain a safety margin from the uncontrolled nucleation temperature (≈4°C below mean recommended)
   - ☐ Set LyoCoN vacuum between 5–10 mbar

3. **Validation**
   - ☐ Evaluate impact on critical quality attributes
   - ☐ Compare against uncontrolled nucleation
   - ☐ Confirm reproducibility across multiple batches

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