ControLyo™
Nucleation On-Demand Technology

PRAXAIR

Technology Background

Lyophilization is the preferred method for drying sensitive, high-value, sterile products. The lyophilization process consists of three steps: freezing, primary drying, and secondary drying. Despite several advances in freeze-drying equipment, the freezing step remains uncontrolled due to the normally random nature of nucleation (i.e., beginning of freezing). In a typical pharmaceutical lyophilization process, the contents of individual vials nucleate over a broad range of temperatures, usually spanning 5-20°C below the formulation's freezing point. This phenomenon negatively impacts lyophilization processes in several ways.

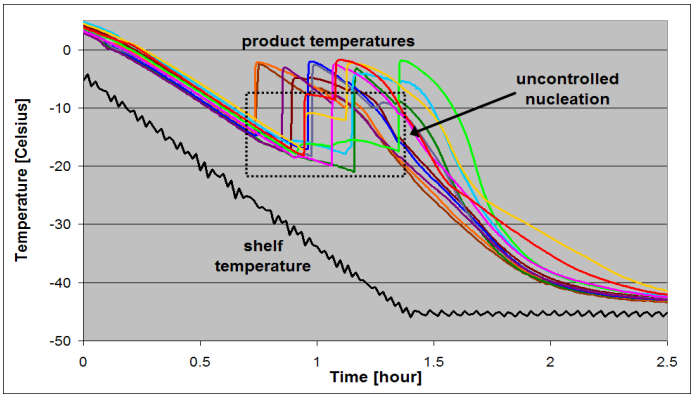
Adverse Effects of Uncontrolled Nucleation

Manufacturing Cost and Capacity	Colder nucleation creates smaller ice crystals – slows drying rate
	Longer cycles require more capacity and have higher operating costs
Product Yield	Resulting ice structures can damage certain API's
	Improper freezing exacerbates vial cracking
	Risk of product loss increases with longer cycles
Product Quality	Vial-to-vial uniformity is impossible
	Poor homogeneity or cosmetic elegance for certain product cakes
	Lack of process control not aligned with FDA QbD initiative
Cycle Development	Complex formulations and conservative cycles help mitigate nucleation problems
	Unpredictable process scale-up and transfer

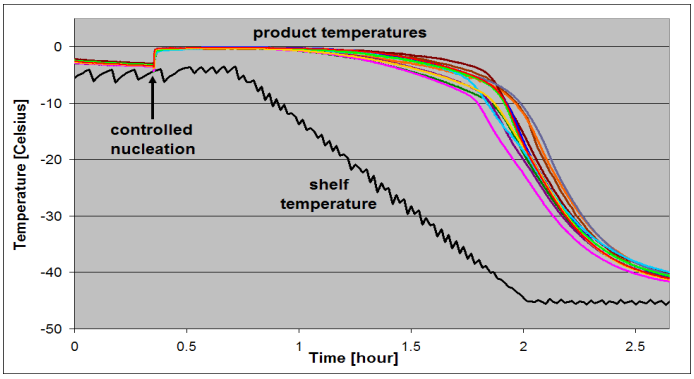
Praxair's Method for Controlling Nucleation

Praxair has developed a novel and scalable method for controlling nucleation of the freezing step in commercial lyophilization. Using Praxair's patent-pending process technology, the nucleation temperature of a solution can be precisely controlled, in many cases to within 1°C of the solution's freezing point. This revolutionary innovation addresses the adverse effects summarized above. Praxair's technology does not require any changes to existing drug formulations and can be retrofitted to most freeze-dryers with minimal impact on established lyophilization protocols.

Uncontrolled Nucleation



Controlled Nucleation



Learn More

Praxair, Inc., a global Fortune 300 firm, is the largest industrial gases company in North and South America and one of the largest worldwide, with operations in 44 countries and over $10.8 billion in sales. The company produces, sells, and distributes atmospheric, process, and specialty gases; supplies high-performance surface coatings; and provides technology solutions to a wide array of industries. Praxair's BioPharma team is committed to supporting the pharmaceutical and biotechnology industries with a broad range of products, services, and technologies that reduce costs and improve productivity from discovery to scale-up to manufacturing. We develop and offer unique, patented solutions to diverse needs in areas such as microbial fermentation, mammalian cell culture, lyophilization, cryogenic cooling, cryopreservation, MRI service, and environmental emission and waste controls. Please visit www.praxair.com for more information.

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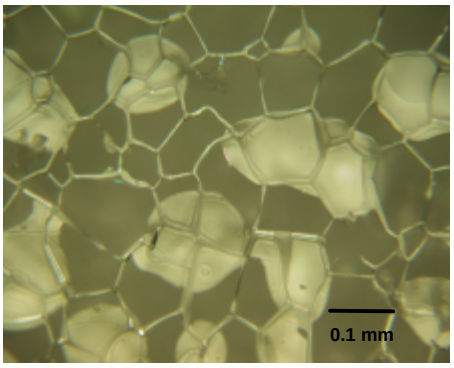
Nucleation On-Demand Technology

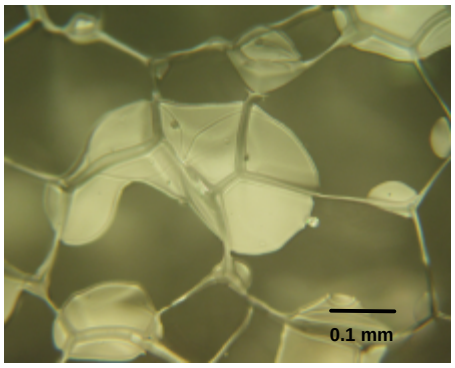
It is generally recognized that smaller ice crystals reduce the primary drying rate as mass transfer is limited through the small pores left behind by the sublimating ice crystals. Previous studies have estimated that for every degree increase in nucleation temperature there is a 1-3% decrease in drying time. It is also recognized that larger surface areas contribute to protein aggregation, which occurs on the ice surface.

Uncontrolled Nucleation



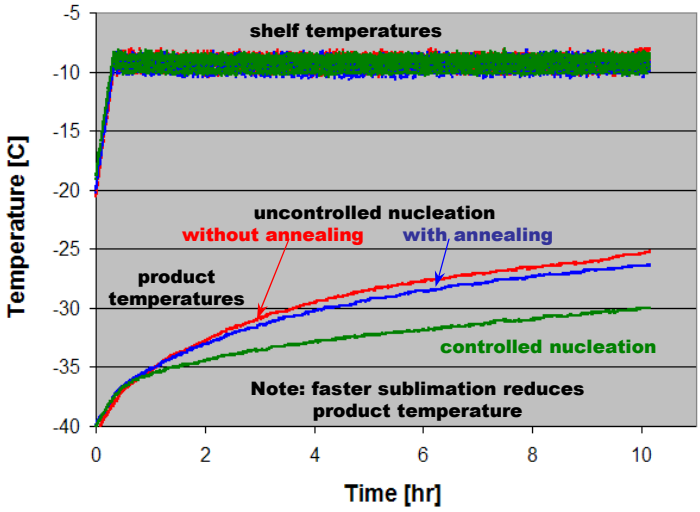
surface area = 1.2 m^2/g pore diameter = 50 µm

Controlled Nucleation



surface area = 0.6 m^2/g pore diameter = 120 µm

Faster Drying, More Uniform Product, Less Vial Cracking



Case Study:

- 3 wt% mannitol solution (3 mL fill in 10 mL vials)
- Controlled nucleation performed at -2 to -3°C
- Uncontrolled nucleation occurred at -8 to -21°C
- Freeze at -45°C for 3 hours
- Anneal at -9°C for 5 hours
- Ramp rates at 0.5°C/min

Nucleation	Uncontrolled		Controlled
Annealing	No	Yes	No
Residual Moisture @ 11 hours [%]	45.3	40.6	36.7
Moisture Standard Deviation [%]	4.6	3.6	2.1
Drying Rate Increase [%]	0	10	19
Product Temp @ 11 hours [°C]	-25.2	-26.4	-30.0
Broken Vials [%]	11	6	0

Reduced Protein Aggregation

Repeated tests have demonstrated that Praxair's controlled nucleation technology has been effective at reducing protein aggregation and improving product activity. These effects have been explored using the model protein lactate dehydrogenase (LDH) with dynamic light scattering (DLS), size exclusion chromatography, and enzyme activity assays. LDH sourced from two different vendors was combined at a concentration of 1, 0.25, or 0.05 mg/mL with either 12.5 or 100 mM citrate (pH 7.5) or Tris (pH 7.5) buffer to make twenty-four different test formulations.

Controlled nucleation reduces incidence of aggregation from 16 of 24 cases (67%) to 6 of 24 cases (25%). Results were generated with DLS

Controlled Nucleation						
	LDH-1			LDH-2		
Buffer	1 mg/mL	200 ug/mL	50 ug/mL	1 mg/mL	200 ug/mL	50 ug/mL
100 mM Tris						
12.5 mM Tris						
100 mM Citrate						
12.5 mM Citrate						

no detectable aggregation aggregation present

Uncontrolled Nucleation						
	LDH-1			LDH-2		
Buffer	1 mg/mL	200 ug/mL	50 ug/mL	1 mg/mL	200 ug/mL	50 ug/mL
100 mM Tris						
12.5 mM Tris						
100 mM Citrate						
12.5 mM Citrate						

Learn More

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