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Effect of Freezing on Lyophilization Process Performance & Drug Product Cake Appearance of Biologics: A Case Study

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Presentation Outline

1. Role of Freezing During Lyophilization Process
2. Impact of Freezing Mechanism on Lyophilized Cake Appearance
3. Role of other Factors: Formulation Composition, Drug Product Presentation, and Lyophilization Process Parameters
4. Conclusions

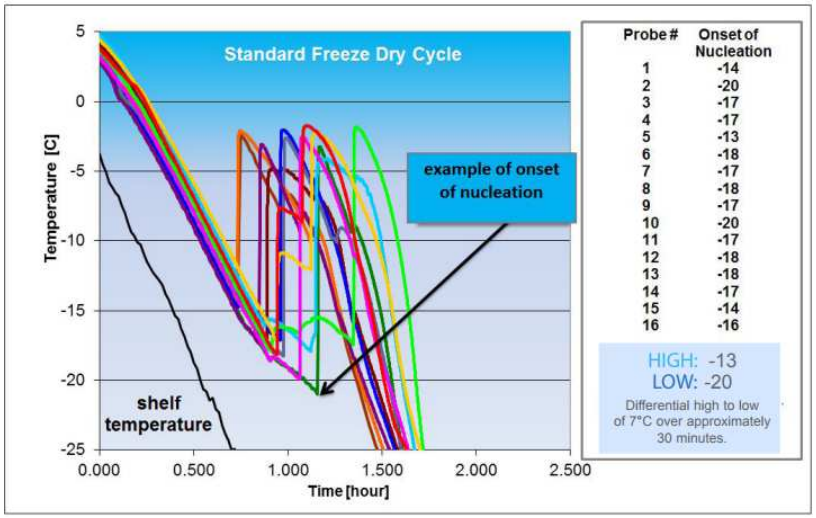
Role of Freezing During Lyophilization Process

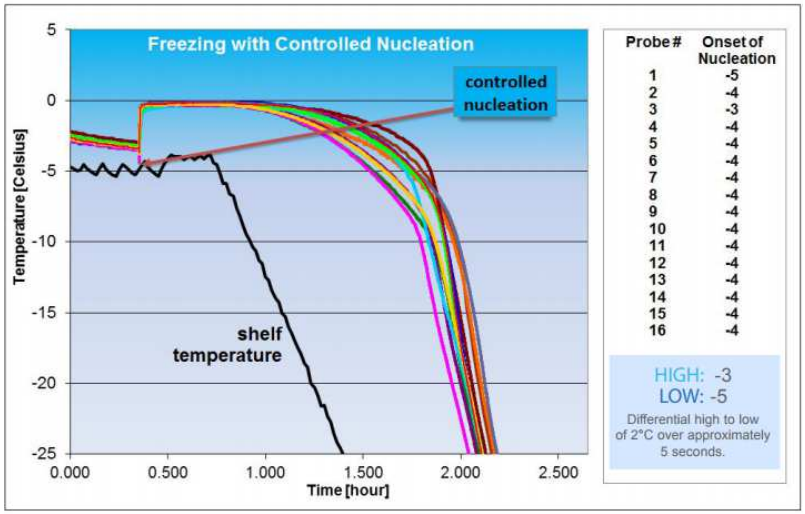
- Freeze-drying is commonly used to achieve acceptable shelf life for an otherwise unstable products.
  - Roughly half of all injectable products are freeze-dried solids*
- Freeze drying is usually carried out in three steps:
  - ***Freezing: conversion of water into ice***
  - Primary drying: removal of ice by sublimation at low temperature and pressure
  - Secondary drying: removal of unfrozen water
- Freezing has received significant attention in the recent past
  - Impact on process performance, e.g., subsequent steps of primary and secondary drying
  - Impact on product quality, e.g., physical stability, cake appearance, residual moisture, reconstitution time

*Schwegman et. al. Pharm Dev Technol. 2005;10:151-173.

Role of Freezing During Lyophilization Process

- During freezing, the ice nucleation temperature (degree of supercooling) is a potentially important parameter
  - Challenge: random and stochastic nature of ice nucleation resulting in intra- and inter-batch heterogeneity
  - Controlled ice nucleation can significantly reduce the heterogeneity and randomness in ice nucleation temperature

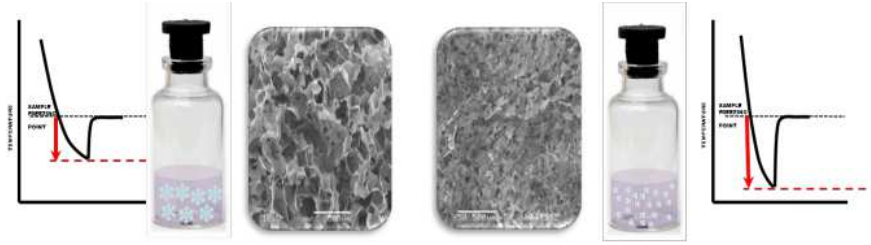




Role of Freezing During Lyophilization Process

- Annealing is often a common approach during freezing to mitigate heterogeneity in ice nucleation temperature
  - In general, reduces primary drying time
  - Crystallizes crystallizing excipients in the formulation.
  - May introduce additional difficulties in some cases (e.g., product quality impact due to conformational changes in protein structure or amorphous-amorphous phase separation).

- In addition to annealing, other freezing mechanisms for better control of ice nucleation temperature have become available in the recent past.
  - Controlled ice nucleation techniques



- **Low degree of supercooling:**
- Larger ice crystals
- Larger pores in the dried matrix
- Decrease of R_p

- **High degree of supercooling:**
- Smaller ice crystals
- Smaller pores in the dried matrix
- Increase of R_p

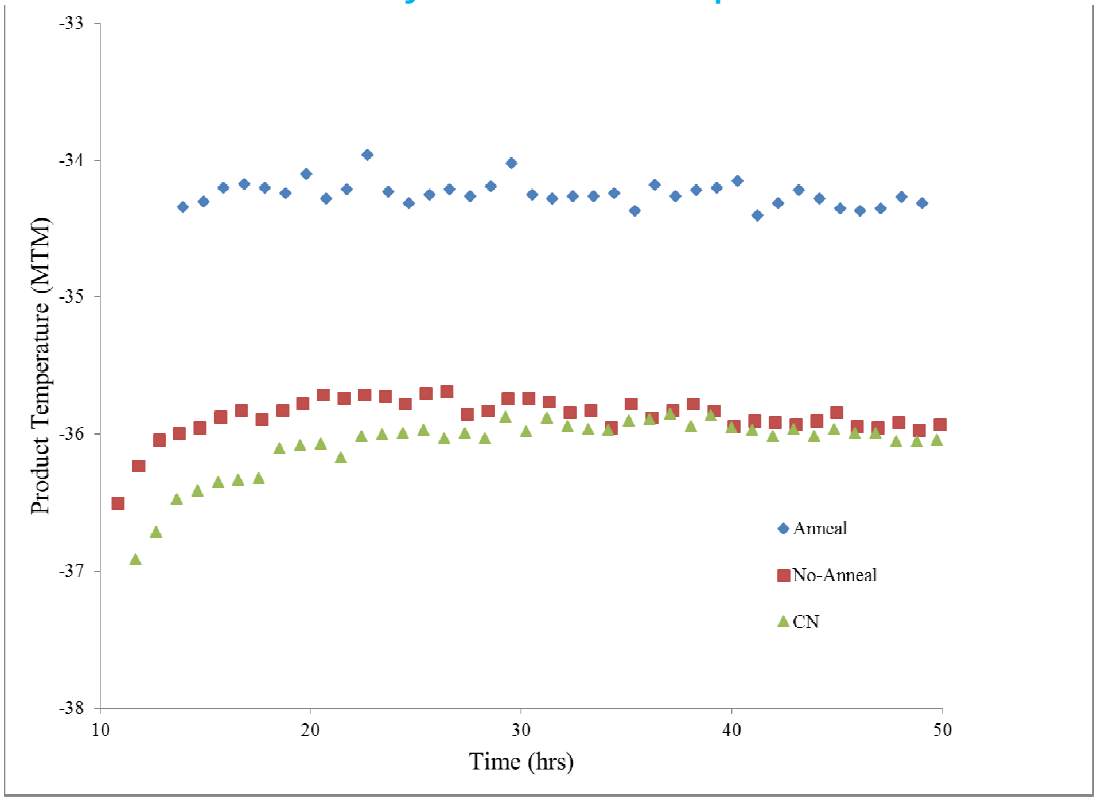
- Here (via a case study) we have evaluated the impact of freezing mechanisms on cake appearance

Impact of Freezing Mechanism: Case Study

- Model Formulation (Tg' ~ -34°C): 10 mg/mL protein X (glycoprotein, ~75 kDa) in a citrate-based buffer containing trehalose, arginine/arginine-HCl mixture, and PS-80
- 3 cycles with differences in only the freezing step
  - SP Scientific Lyostar 3 unit at 20% total load; 3.5 mL in 10 mL vials

Process Parameter	Anneal Cycle	No Anneal Cycle	Controlled Nucleation (CN) Cycle
Ramp to Freezing	1 °C/min		
Freezing temp/hold time	-40 °C/4 hrs		
Annealing temp/hold time	***-15 °C/2 hrs***	***NA***	***NA***
CN temp/hold time	***NA***	***NA***	***-8 °C/4 hrs***
Ramp to primary drying	0.1 °C/min		
Primary drying temp/pressure/hold time	-30 °C/100 mTorr/Pirani		
Ramp to secondary drying	0.3 °C/min		
Primary drying temp/pressure/hold time	40 °C/100 mTorr/6 hrs		

Case Study-Product Temperature



- Monitored using thermocouples and determined using MTM methodology.
- During steady state, product temperature similar for No Anneal and CN cycles at ~ 35.5°C, warmer by ~ 1°C for Anneal cycle

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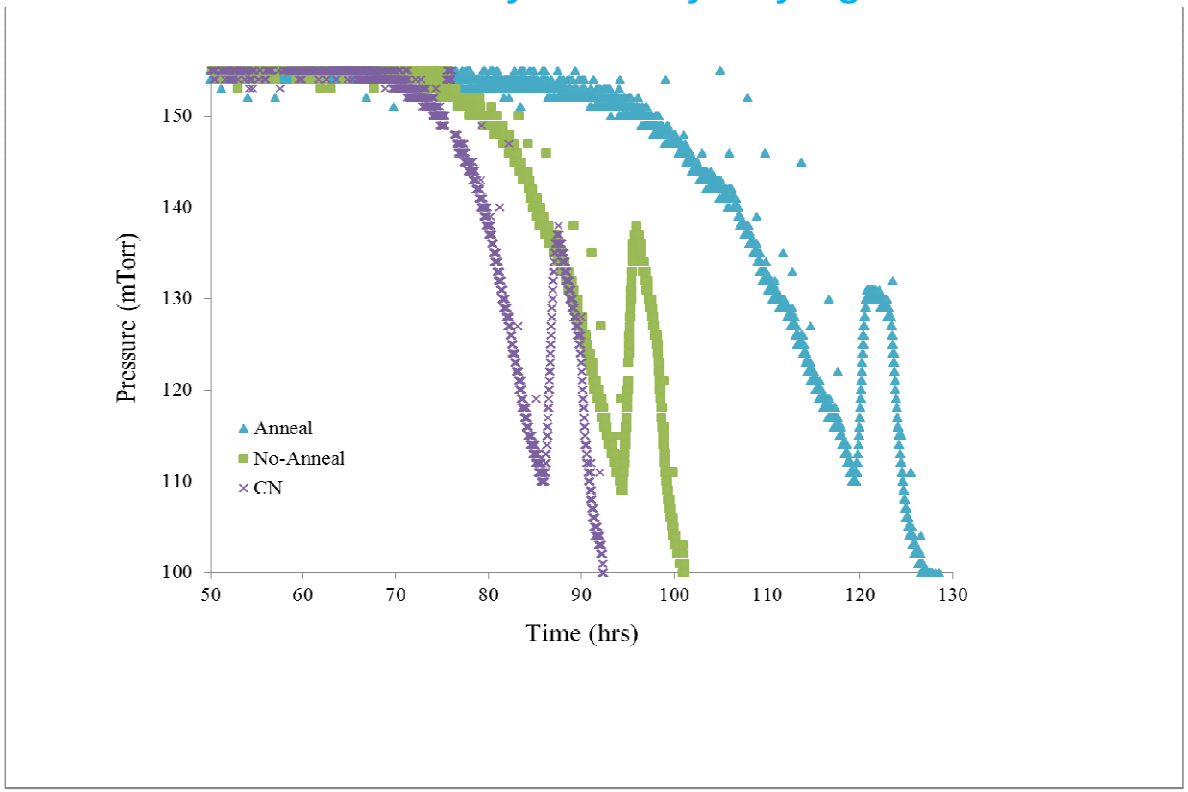
Case Study-Primary Drying Time



- Anneal (~120 hrs) > No Anneal (95 hrs) > CN (85 hrs)
- Differences observed exclusively due to differences in freezing mechanism (all other parameters the same)
- Difference in slopes of the Pirani pressure drop at ~ 4, 2.5, and 1.5 mTorr/hr for Anneal, No Anneal, and CN cycles
  - Batch homogeneity: CN > No Anneal > Anneal.

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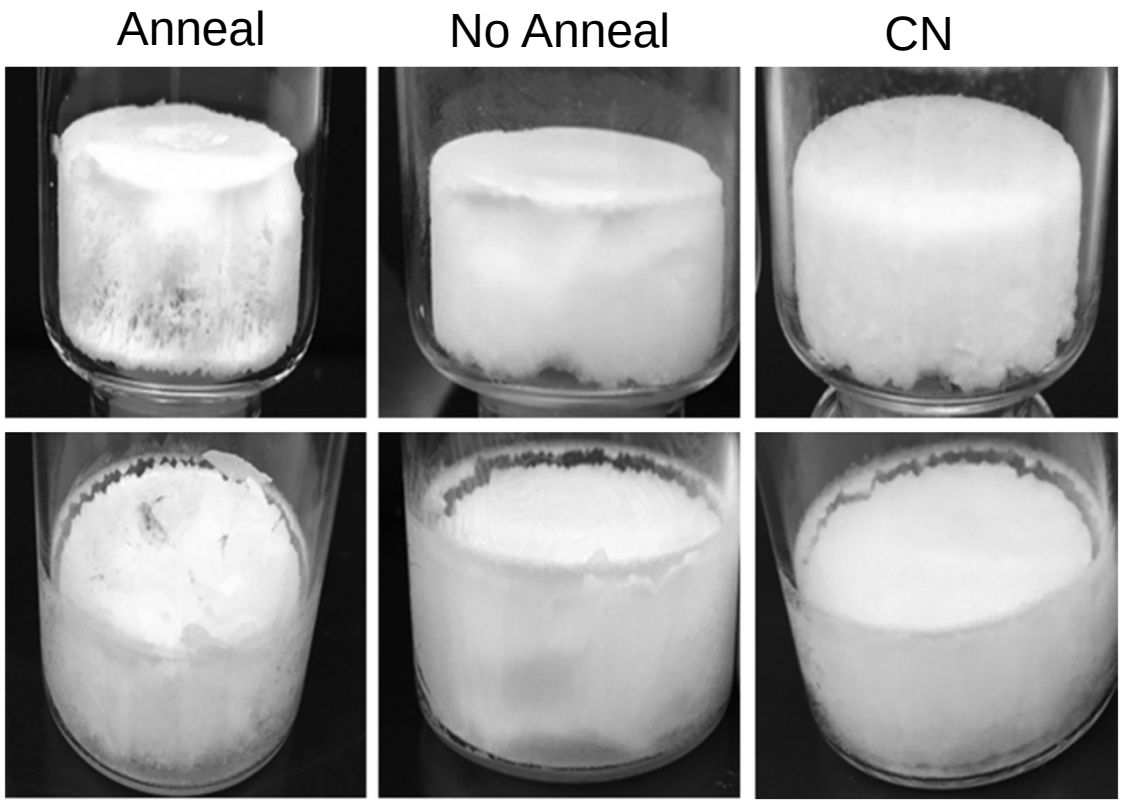
Case Study-Cake Appearance



- Anneal: poorest quality, extensive cake shrinkage at the bottom, cracks, collapse, and high porosity, skin formation at the top
- No Anneal: improved appearance, still partial collapse at the bottom, no visible skin formation
- CN: elegant cakes with no cake defects.

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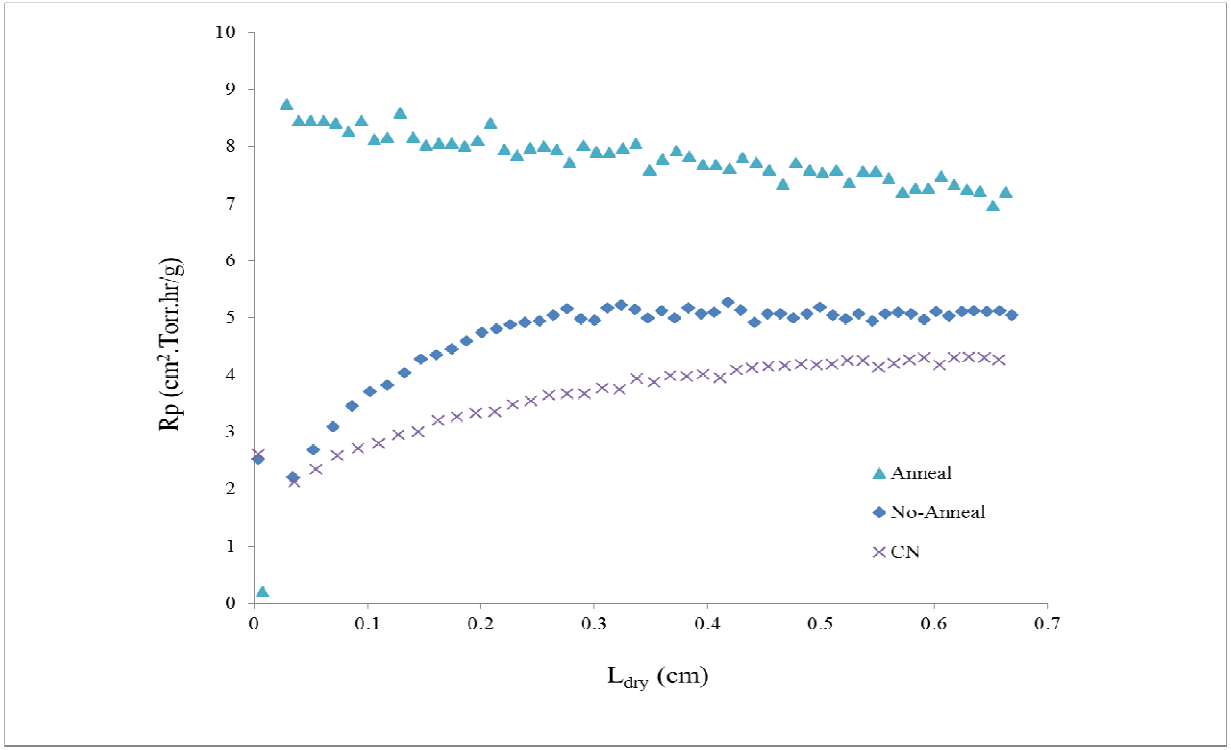
Case Study-Product Resistance



- Anneal ~ 7.5, No Anneal ~ 5, CN ~ 4 cm^2.torr.hr/g dried layer resistance
- Lower product resistance correlates with shorter primary drying time
- Anneal: high product resistance at the start of drying followed by a decrease; characteristic of skin formation followed by micro-collapse/collapse during primary drying

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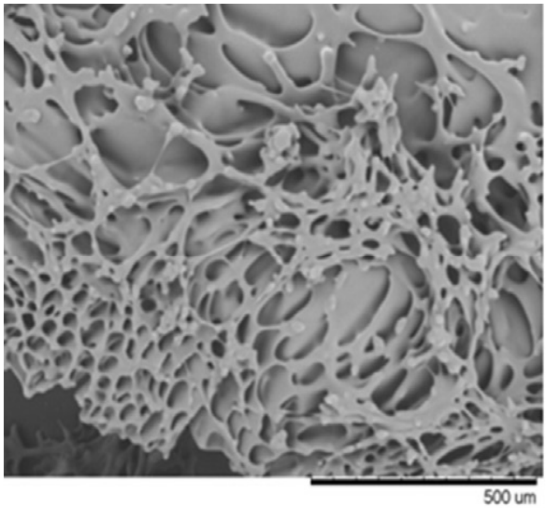
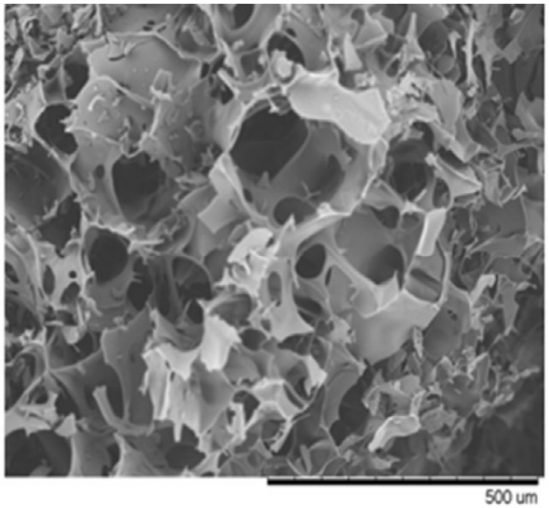
Case Study-Cake Morphology by Scanning Electron Microscopy

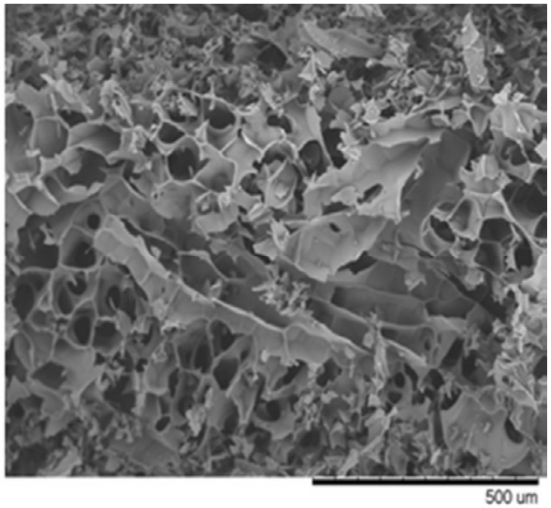
Anneal



No Anneal



CN



- Significant differences in pore structure when comparing Anneal to No Anneal and CN cycles
- No Anneal and CN exhibited similar, more typical pore structures; Anneal showed a distinct layer at the top, evident by the exhibited solid sheet (i.e., closed pore structure)
- Aligned with cake appearance observations with skin formation from Anneal cycle.

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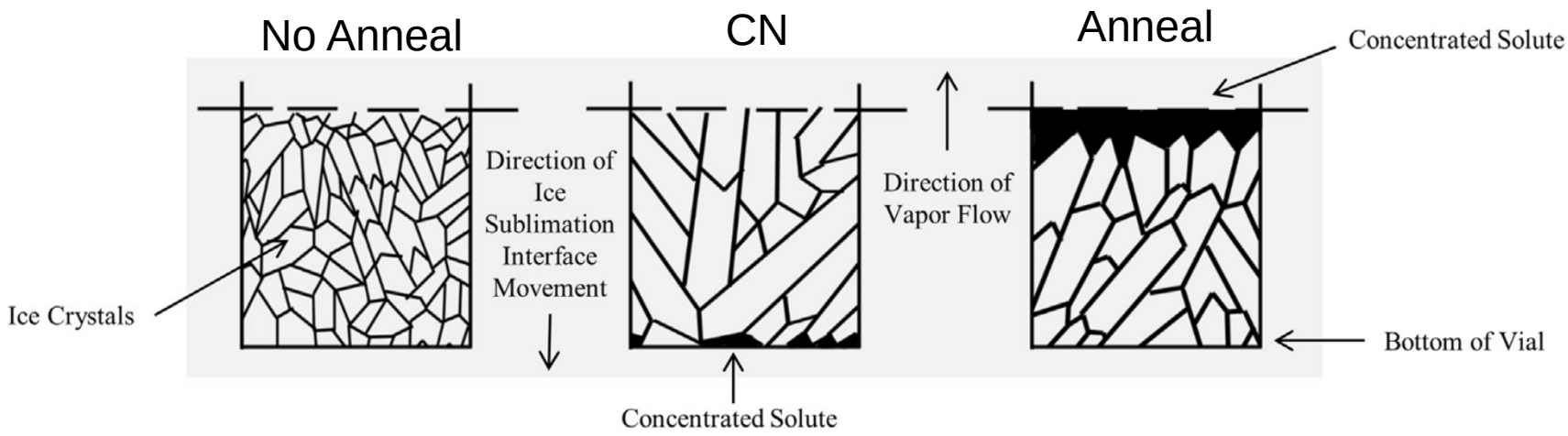
Case Study-Product Quality Impact

- Residual moisture low ~ 1% across all three cycles.
- Similarly fast reconstitution time < 2min across all three cycles.
- Stability: evaluated for 1 month at 40°C
  - No change in product quality pre- and post-lyophilization as well as on accelerated storage stability
- Overall, no impact on product stability, only impact on lyophilization process performance (e.g., primary drying time)

Proposed Mechanism of Skin Formation



– Annealing: crystals grow bottom to top. The highly concentrated solutes trapped between ice crystals are expelled via a convective flow towards the top, resulting in skin formation.

– Controlled ice nucleation: ice first nucleates at the top followed by a downward propagation, resulting in uniform ice crystal growth and homogeneous distribution of solutes within the interstices.
  - In extreme cases, if a skin forms, it should form at the bottom of the vial (not observed in this case study)

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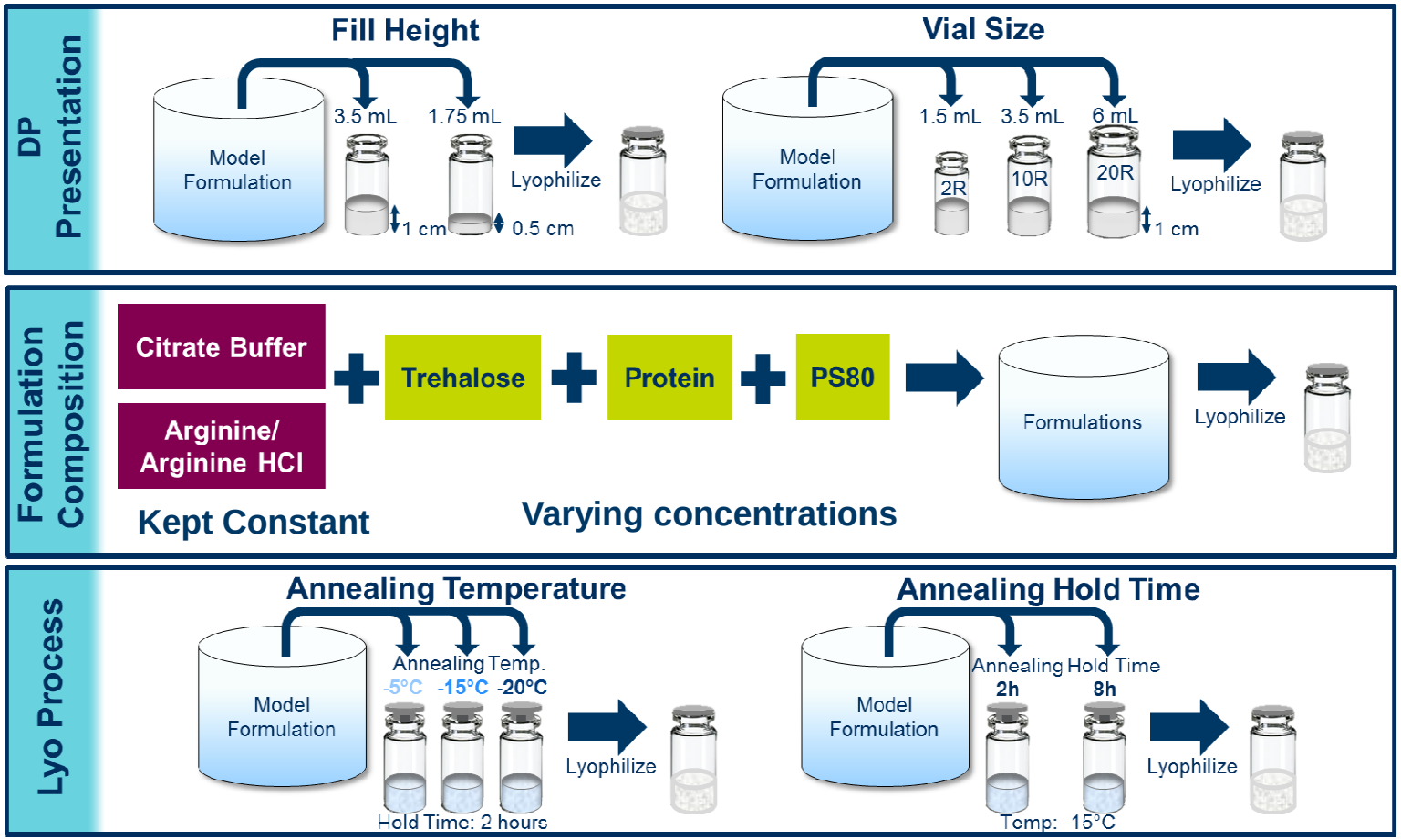
Role of DP Formulation & Presentation, and Lyo Process



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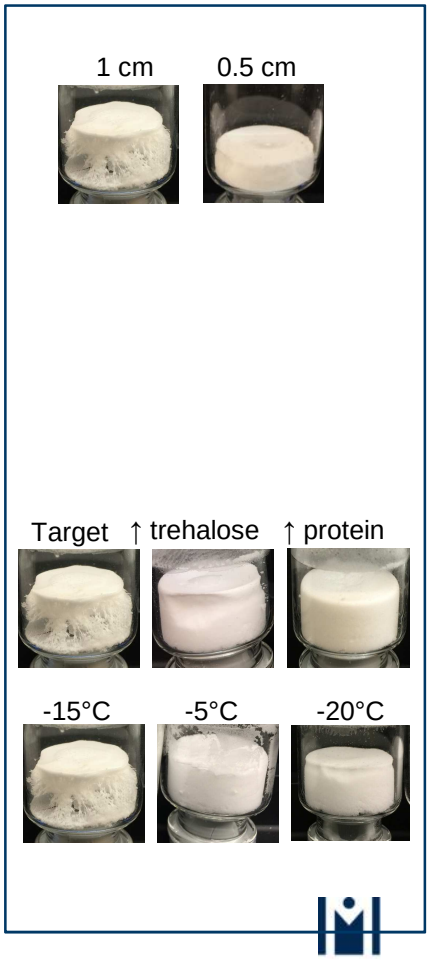
Role of DP Formulation & Presentation, and Lyo Process*

Parameter	Variable		Outcome
Drug Product Presentation	Fill Height		Decreasing the fill height resulted in elegant lyophilized cakes and improved process efficiency (60% shorter primary drying time)
	Vial Size		Reducing vial size improved process efficiency, although there was no significant improvement in cake appearance.
Formulation Composition	Protein Type		No improvement
	Protein Size		
	PS80 conc		
	Total solids	Trehalose conc	Increasing the total solids resulted in elegant cake appearance.
		Protein conc	
Lyophilization Process	Annealing Temperature (-20, -15, -5°C)		Annealing at -5°C and -20°C improved process efficiency (36% and 23% shorter primary drying time) and resulted in elegant lyophilized drug product
	Annealing Time (2 & 8 hrs)		No improvement



* All studies done with the annealing cycle

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Conclusions

- Although primary drying continues to receive the highest attention from process optimization and scale-up perspectives, the criticality of freezing step in process design, development, optimization, and scale-up is not to be ignored.

- The freezing mechanism can impact process performance and product quality. For the particular case study here:
  - Contrary to common knowledge, annealing resulted in poor process performance and cake appearance
  - Use of controlled ice nucleation resulted in significant improvements including improved cake appearance and reduced primary drying time

- Ongoing work continues to investigate the mechanisms behind skin formation and role of contributing factors such as DP formulation and presentation as well as lyo process parameters on process performance and product quality.
  - Are the observations unique to the formulation matrix examined in this case study?
  - Role of annealing temperature in skin formation
  - Use of more sophisticated analytical tools such as 3D X-ray micro-CT for more in depth characterization of cake morphology as a function of freezing mechanism

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