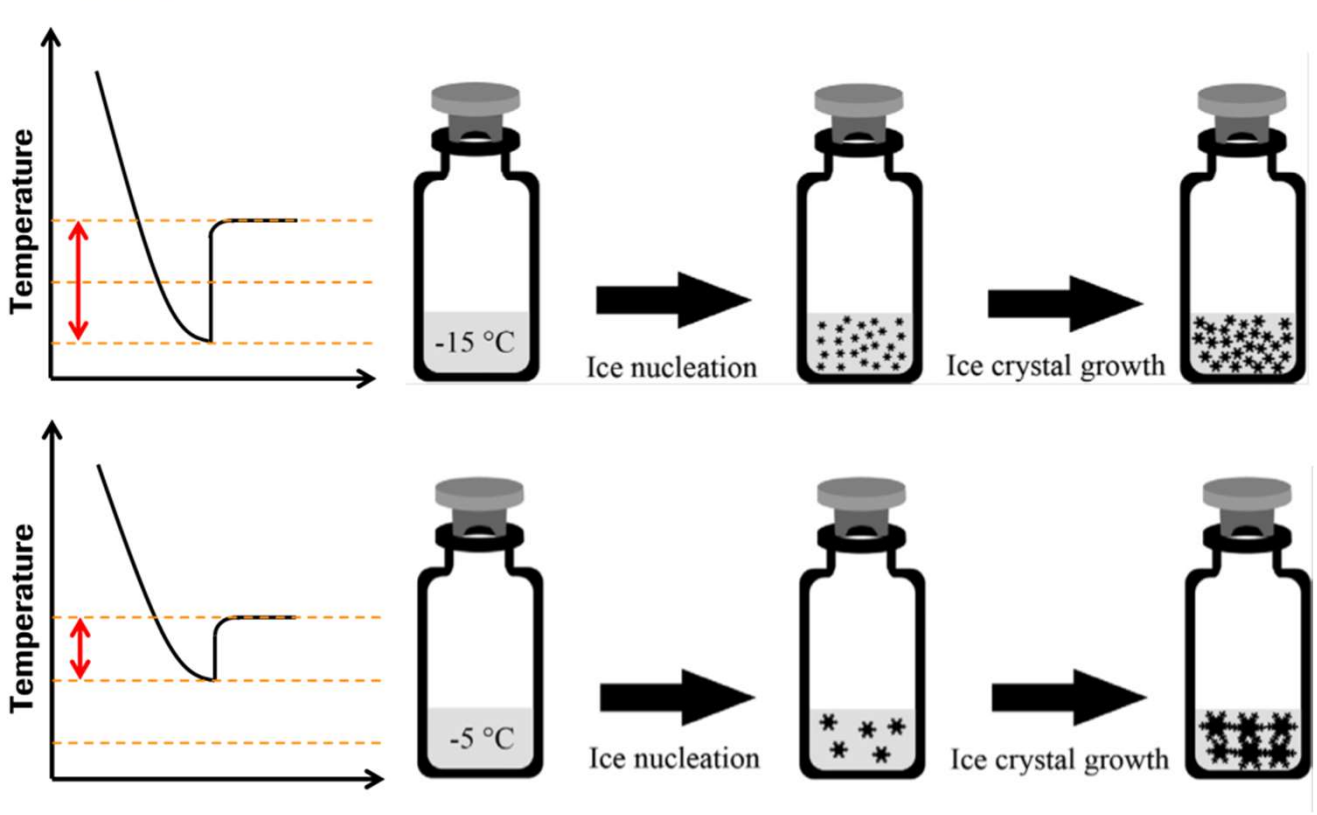
Theory 10

PD Dr. Andrea Allmendinger

CSO, Ten23 Health;
Group leader Pharmaceutical Technology,
University of Freiburg

allmendingerandrea@gmail.com

Controlled nucleation



Low degree of supercooling

Big dentritic ice crystals

Morphology

Drying time

- Increases inter-/intra-batch- and vial-to-vial homogeneity
- Shorter primary drying
- Better stability (?)

Review: Geidobler R, Winter G. Eur J Pharm Biopharm. 2013 Oct;85(2):214-22

Video

Praxair.mp4

Monitoring

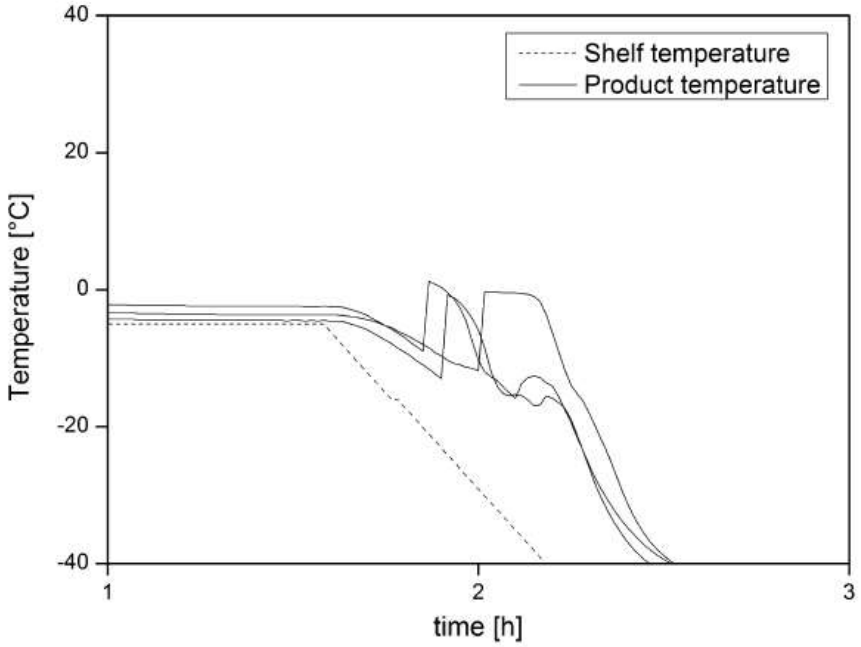


Fig. 1. Typical thermocouple readings for shelf ramp freezing.

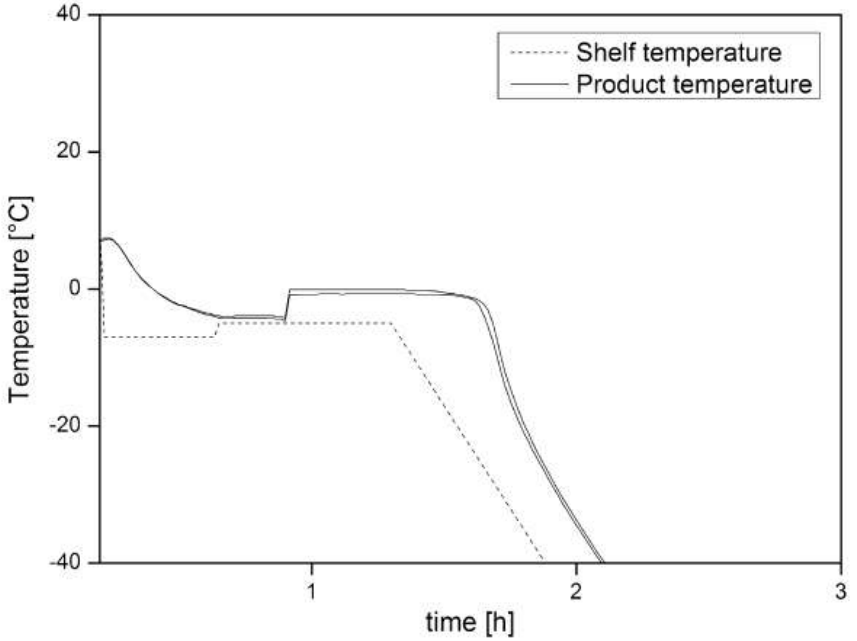
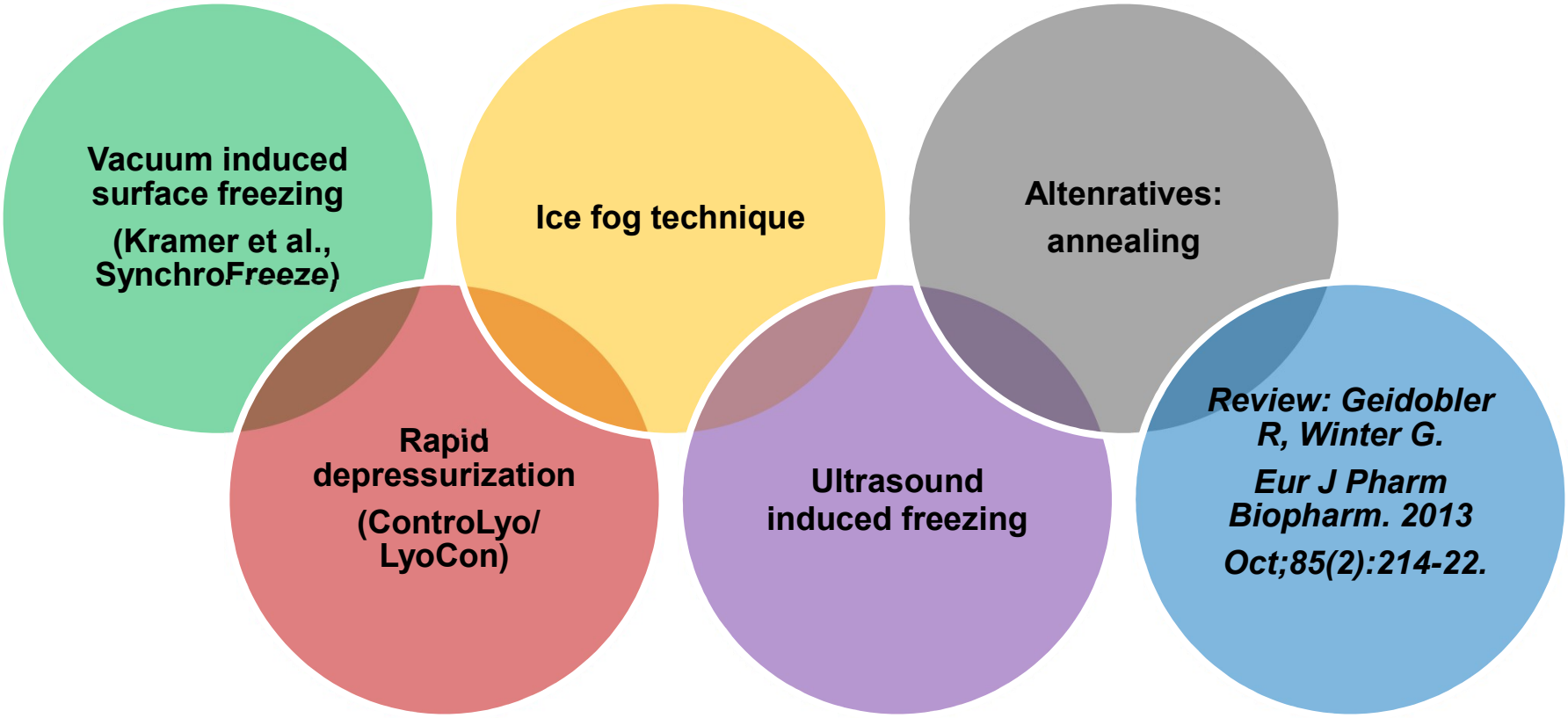


Fig. 3. Thermocouple readings for controlled nucleation at approximately −5 °C followed by 20 min of isothermal hold (unpublished data by the authors).

Review: Geidobler R, Winter G. Eur J Pharm Biopharm. 2013 Oct;85(2):214-22

Methods for controlled nucleation



Controlled Ice Nucleation during Lyophilization

- Comparison of Nucleation Techniques and their Impact on Protein Stability

Andrea Allmendinger and Jake Luoma

Pharmaceutical Development
Roche/Genentech, Basel/San Francisco

Conference Freeze-Drying of Pharmaceuticals and Biologics
Garmisch-Patenkirchen, September 2018

Outline

Roche

I

Background to controlling ice nucleation (CIN)

- Benefits
- Technologies

II

Case-study:

Comparison of nucleation techniques

- Solid state
- Stability
- Challenges

III

Summary and take-home messages

Outline

Roche

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Background to controlling ice nucleation (CIN)

- Benefits
- Technologies

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Case-study:

Comparison of nucleation techniques

- Solid state
- Stability
- Challenges

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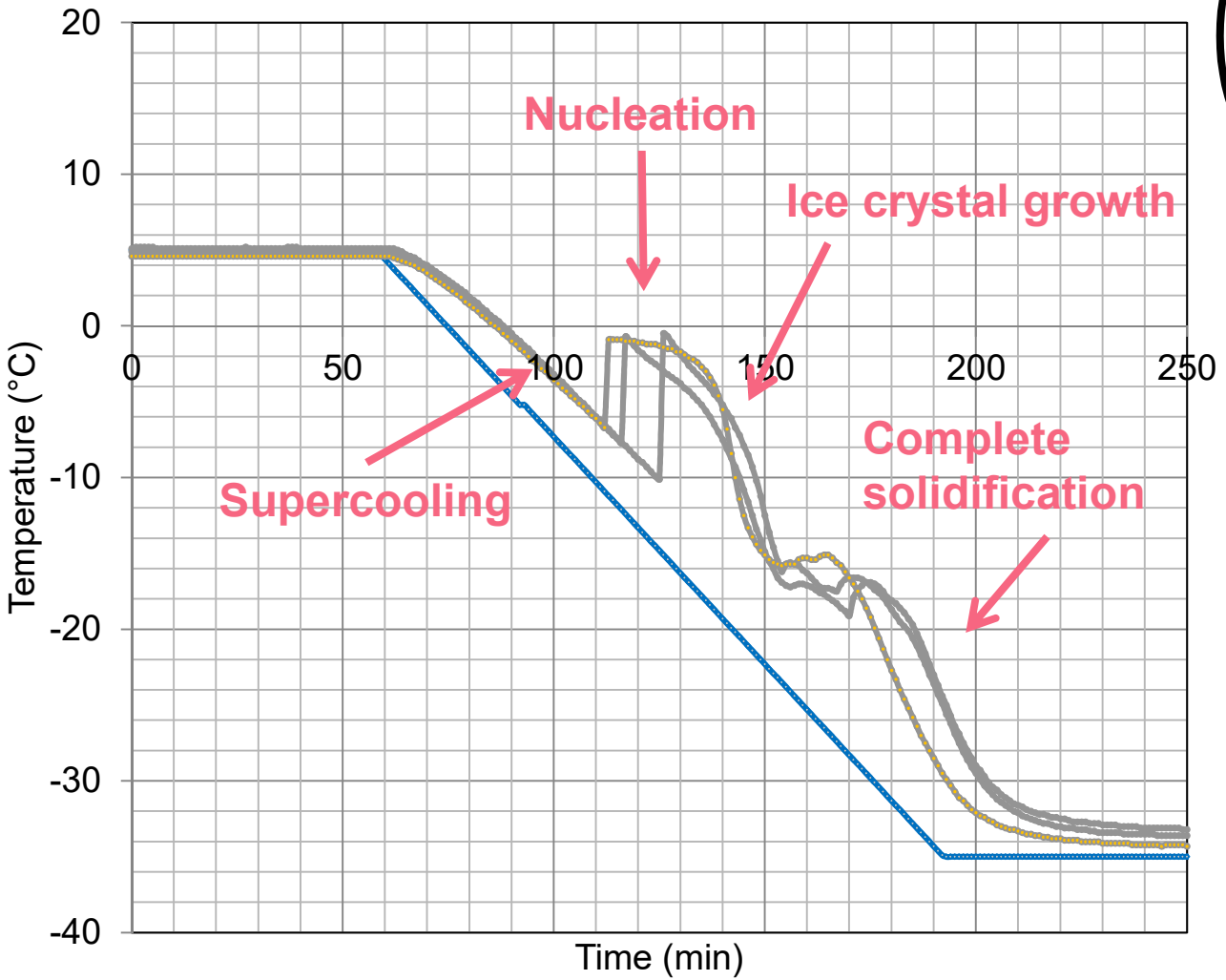
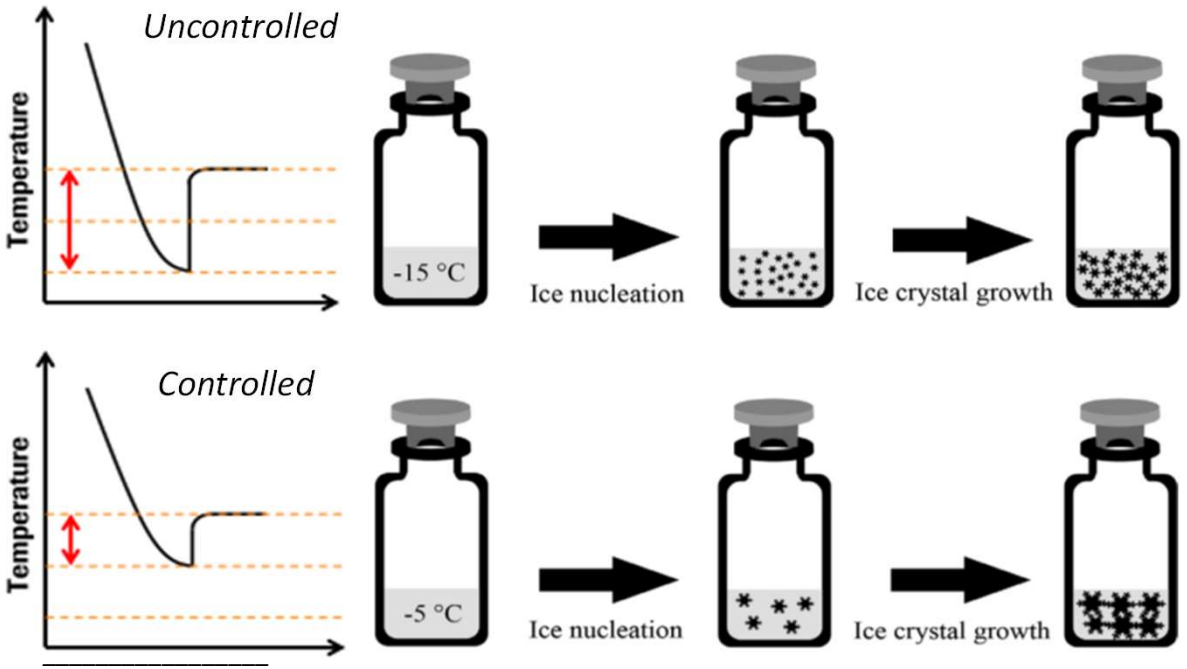
Summary and take-home messages

Standard freezing step



Nucleation temperature impacts cake structure, CQAs, and cycle time



Pros of CIN

+ **Increase inter-vial homogeneity**
+ Shorter primary drying
+ Improved cake appearance

Cons of CIN

- Higher residual moisture
- Intra-vial homogeneity

Geidobler et al.: Controlled ice nucleation in the field of freeze drying: Fundamentals and technology review. Eur J Pharm Biopharm. 85(2):214-22. (2013).

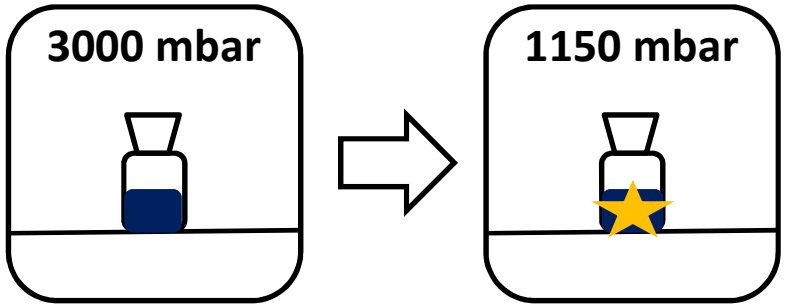
→ Lower vial-to-vial variability reduces scale differences and improves confidence in technical transfers especially for products which are difficult to lyophilize like molecules which are sensitive to moisture or surface area

Technologies for controlling ice nucleation

- Techniques used in the following case study

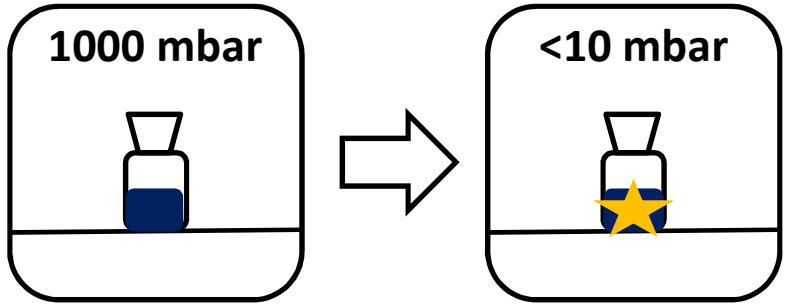
Depressurization

SP Scientific ControLyo®



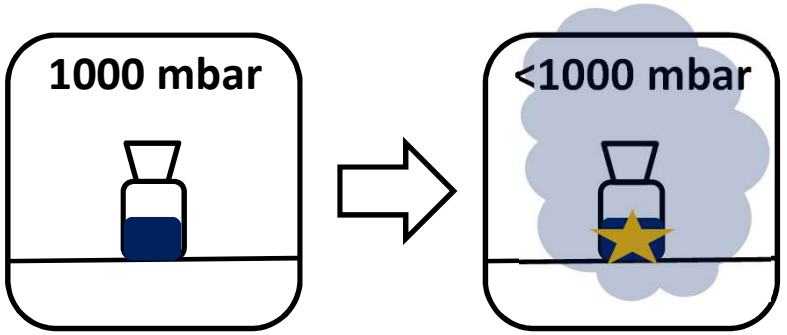
Partial Vacuum

HOF SynchroFreeze™



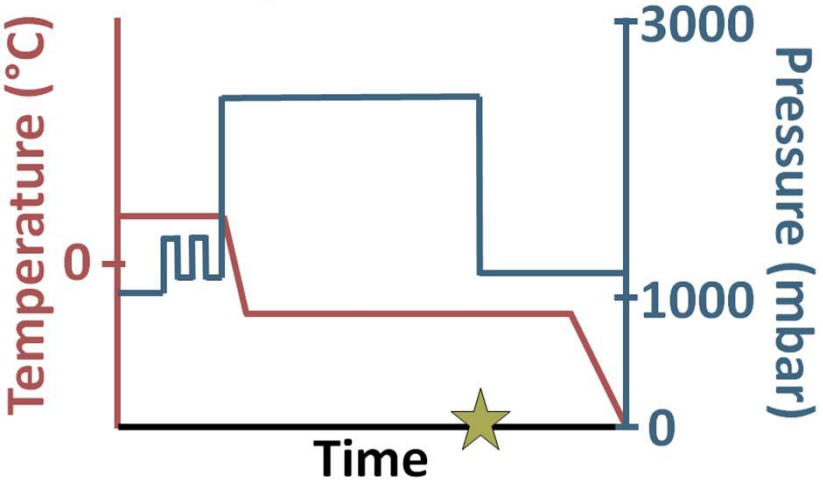
Ice Fog

Linde/IMA VERISEQ®

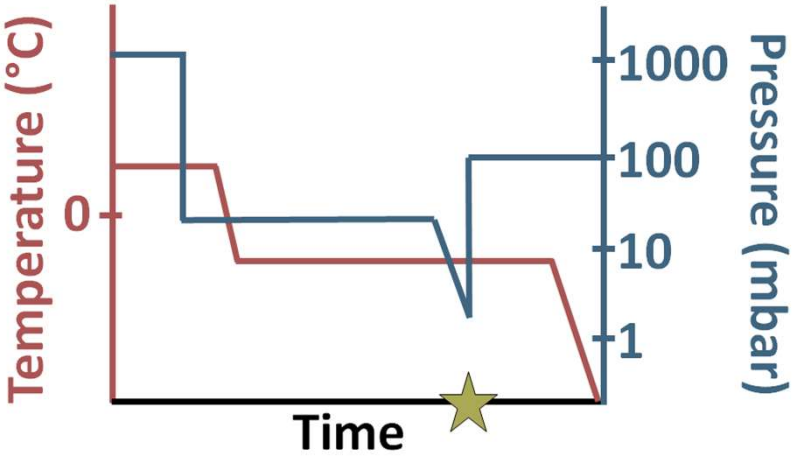


Controlled ice nucleation - Modes of operation

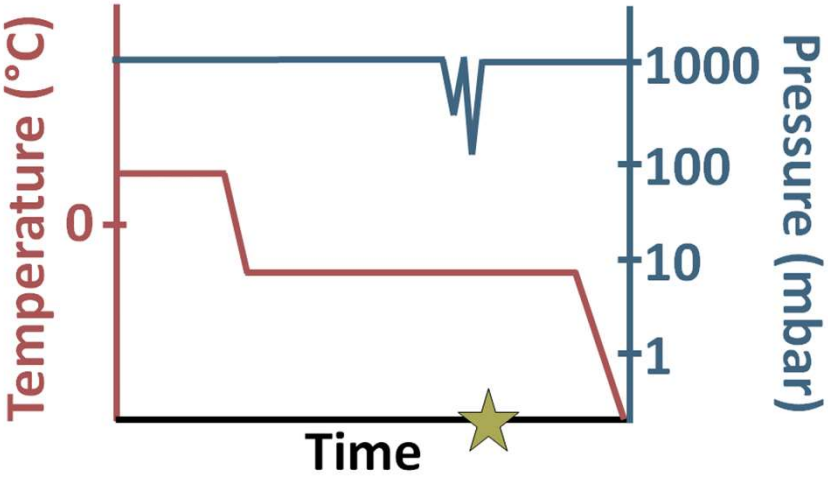
Depressurization



Partial Vacuum



Ice Fog



Nucleation event

Outline

Roche

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Background to controlling ice nucleation (CIN)

- Benefits
- Technologies

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Case-study:

Comparison of nucleation techniques

- Solid state
- Stability
- Challenges

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Summary and take-home messages

Study design & objective

Examining the impact of vial size and formulation

- Determine whether each technology produces comparable drug product when using similar freezing protocols
- Identify any processing limitations under challenging conditions

Formulation #	Type of protein	Protein concentration	Total solid content	Main excipient*	Vial format (cc)	Nominal fill (mL)
1	mAb IgG_1 (148 kDa)	10 mg/mL	9%	240 mM Sucrose	2	1
					20	10
					50	20
2	mAb IgG_1 (148 kDa)	100 mg/mL	18%	240 mM Sucrose	2	1
					20	10
					50	20
3	Enzyme (59 kDa)	2.5 mg/mL	11%	500 mM Arginine Phosphate	6	0.9
					20	10
					50	20

** All formulations contain a formulation buffer and surfactant.*

Nucleation temperatures achieved

Overview of nucleation temperatures for different formulations.

Formulation #	Protein conc.	Total solid content	Vial format (cc)	Nominal fill (mL)	Highest controlled nucleation temperature achieved		
					Depressurization	Partial vacuum	Ice fog
1	10 mg/mL mAb	9%	2	1	Failure to nucleate (UCN)	-5	-5
			20	10	-5	-5	-5
			50	20	-5	-5	-5
2	100 mg/mL mAb	18%	2	1	Failure to nucleate (UCN)	-15	n.p.
			20	10	-5	-15	-5
			50	20	-5	-15	n.p.
3	2.5 mg/mL enzyme	11%	6	0.9	-10	-5	n.p.
			20	10	-5	-5	n.p.
			50	20	-10	-15	-10

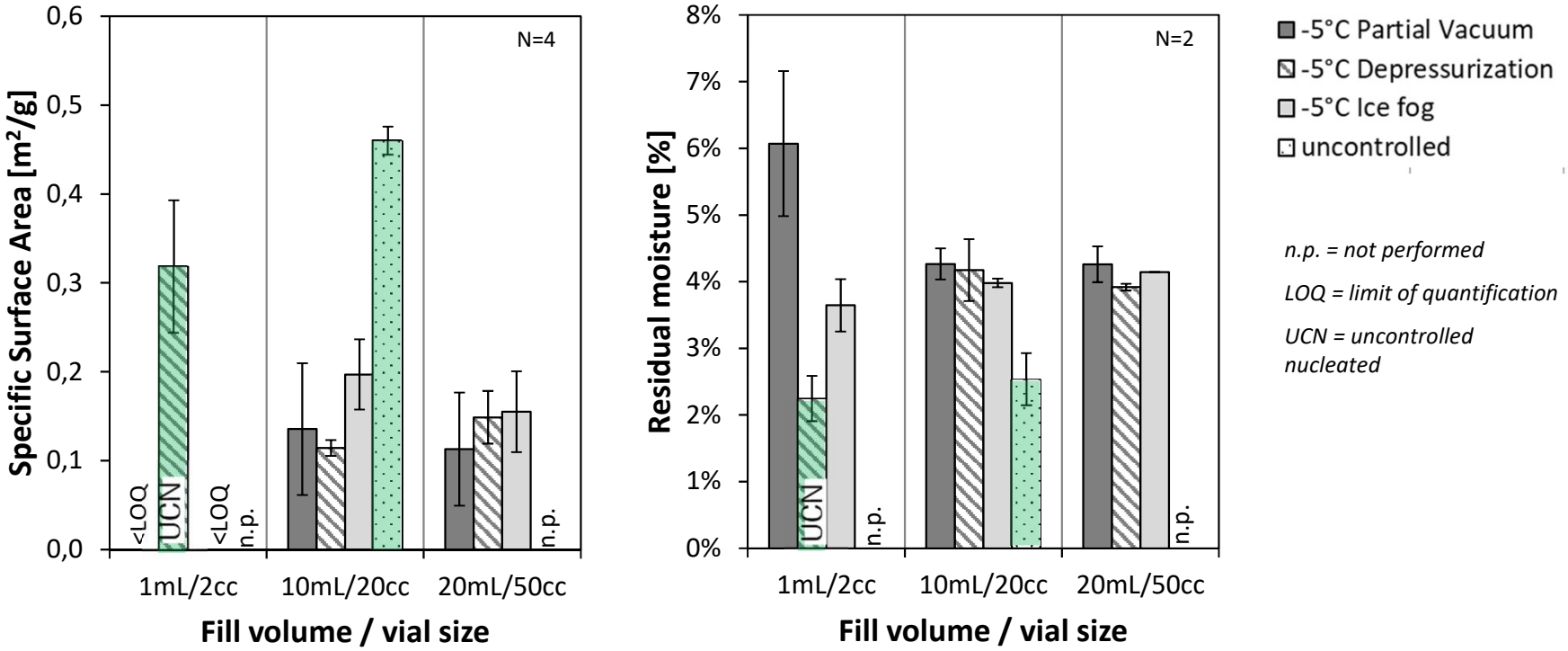
n.p. = not performed, UCN = uncontrolled nucleated

- Depressurization method struggled with 2cc vials
- Partial vacuum method struggled with Formulation 2/3 (high total solids)

Nucleation at the same temperature

– solid state characterization

Formulation 1: 10 mg/mL mAb, nucleation temperature: -5° C



- Nucleation at the same temperature resulted in comparable solid state properties

Nucleation at the same temperature

– cake appearance

Formulation 1: 10 mg/mL mAb, nucleation temperature: -5° C (20cc vial)

Partial vacuum	Depressurization	Ice fog	Uncontrolled

- Nucleation at the same temperature resulted in comparable visual cake structure. No denting was observed with controlled nucleation.
- There were no significant changes on (accelerated) stability (SEC/IEC 5/25/40° C 1Y)

Nucleation temperatures achieved

Overview of nucleation temperatures for different formulations.

Formulation #	Protein conc.	Total solid content	Vial format (cc)	Nominal fill (mL)	Highest controlled nucleation temperature achieved		
					Depressurization	Partial vacuum	Ice fog
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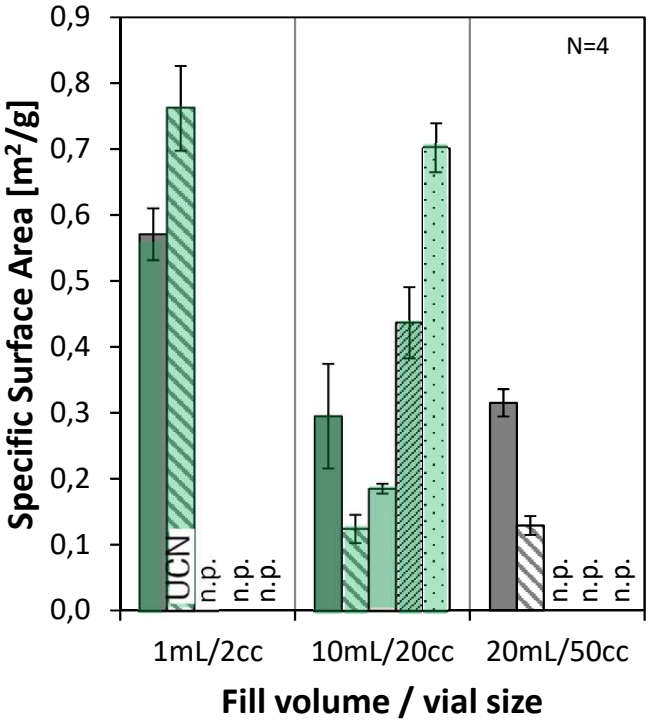
n.p. = not performed, UCN = uncontrolled nucleated

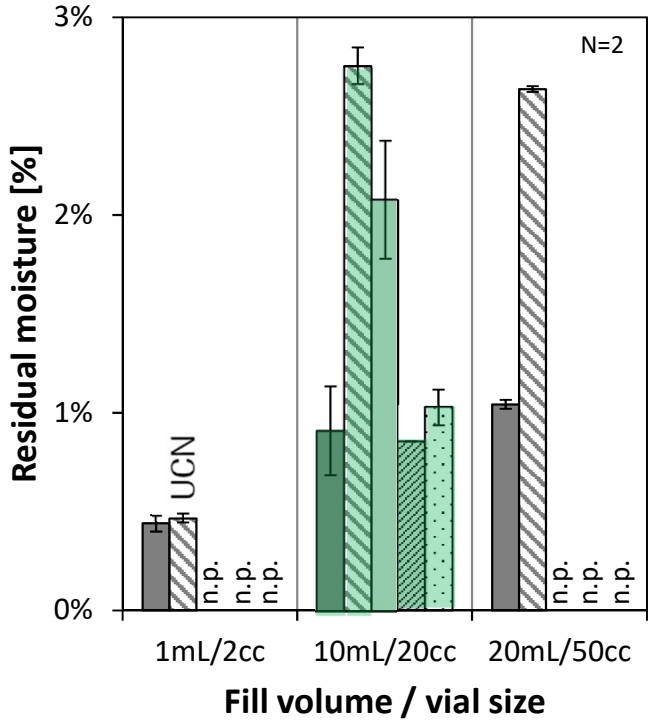
- Depressurization method struggled with 2cc vials
- Partial vacuum method struggled with Formulation 2/3 (high total solids)

Nucleation at different temperatures

– solid state characterization

Formulation 2: 100 mg/mL mAb, nucleation temperature: -5° C and -15° C





-15°C Partial Vacuum
-5°C Depressurization
-5°C Ice fog
-15°C Ice fog
Uncontrolled

n.p. = not performed

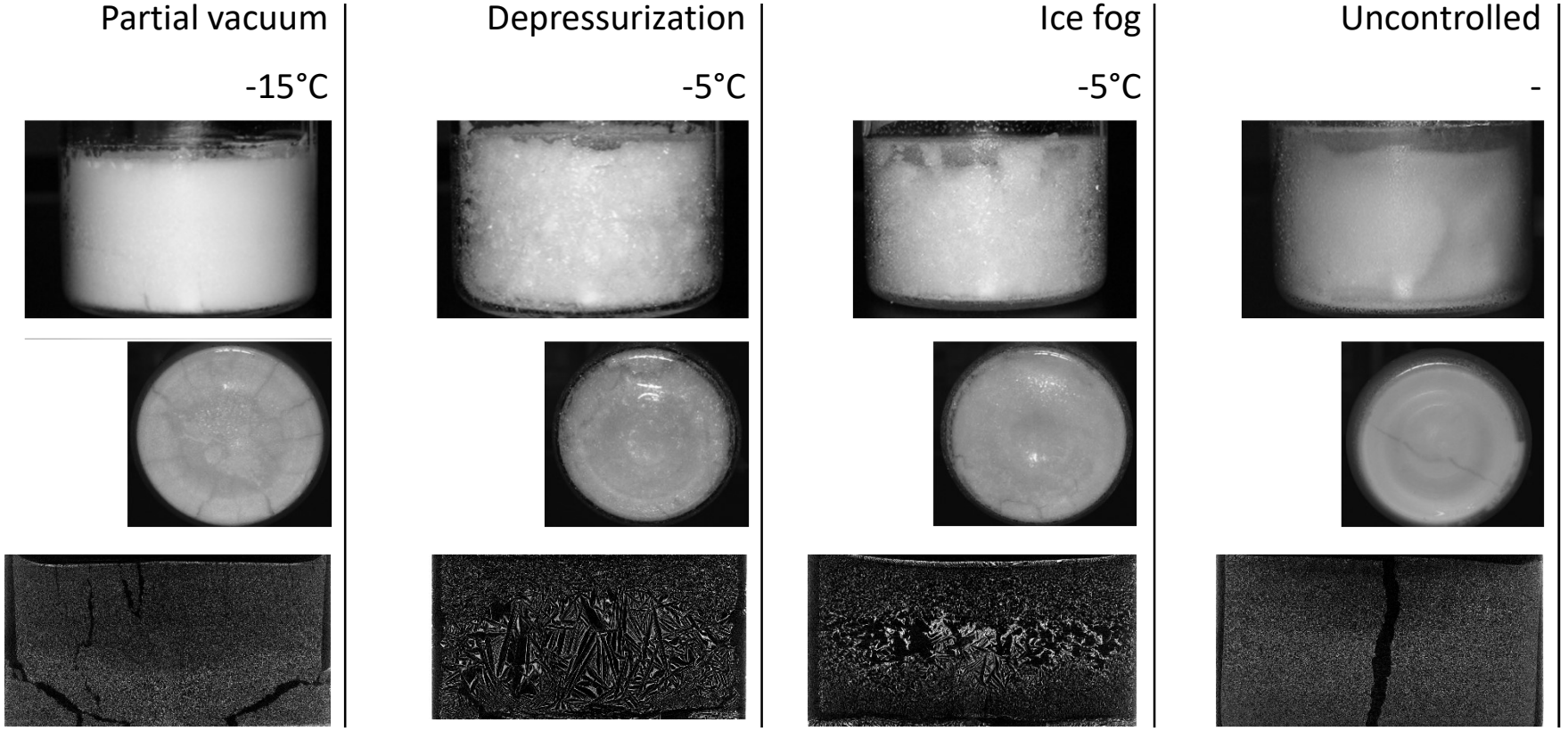
UCN = uncontrolled nucleated

- Nucleation ten degrees apart resulted in large changes to solid state properties

Nucleation at different temperatures

– cake appearance and macroscopic cake structure

Formulation 2: 100 mg/mL mAb, nucleation temperature: -5° C and -15° C

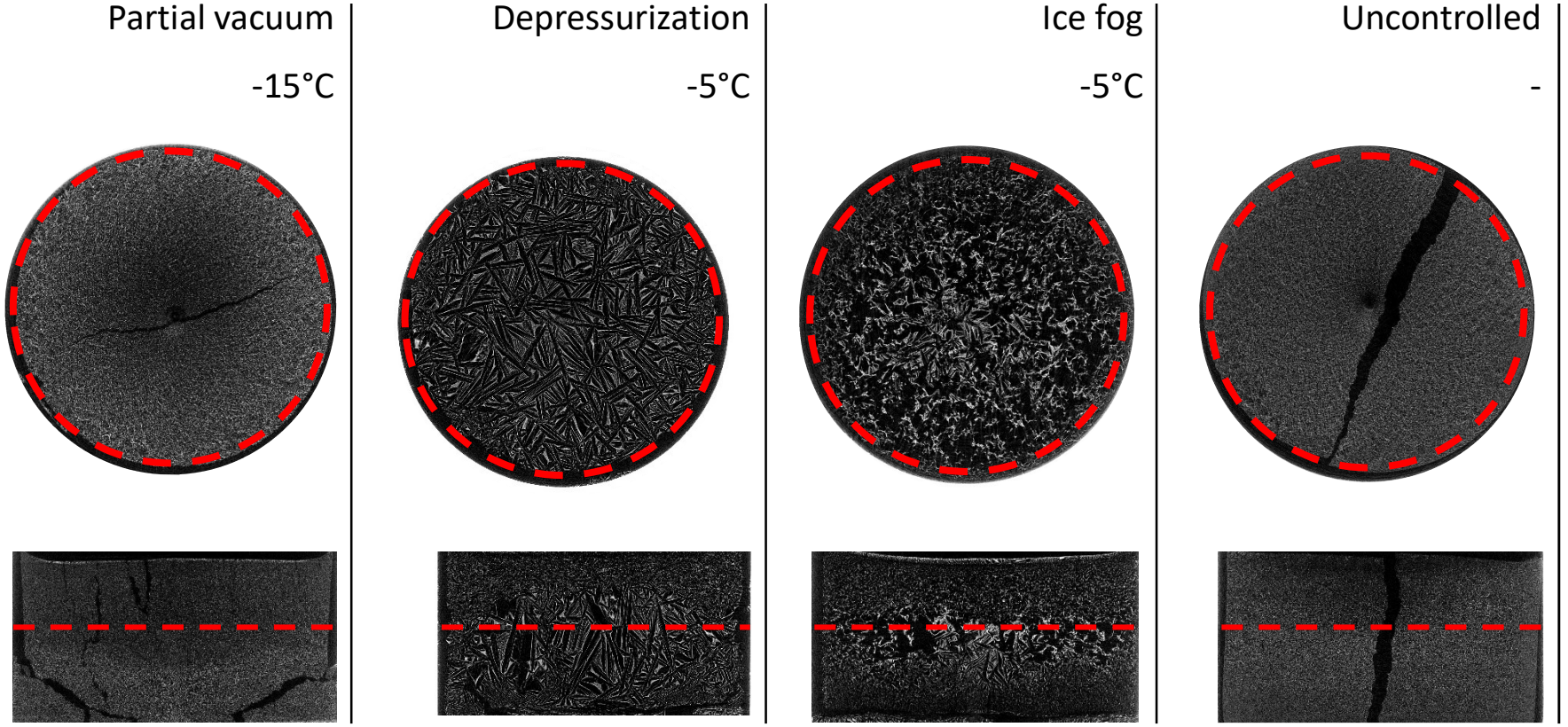


- Nucleation ten degrees apart resulted in large changes in cake structure and macroscopic cake structure

Nucleation at different temperatures

– cake appearance and macroscopic cake structure

Formulation 2: 100 mg/mL mAb, nucleation temperature: -5° C and -15° C

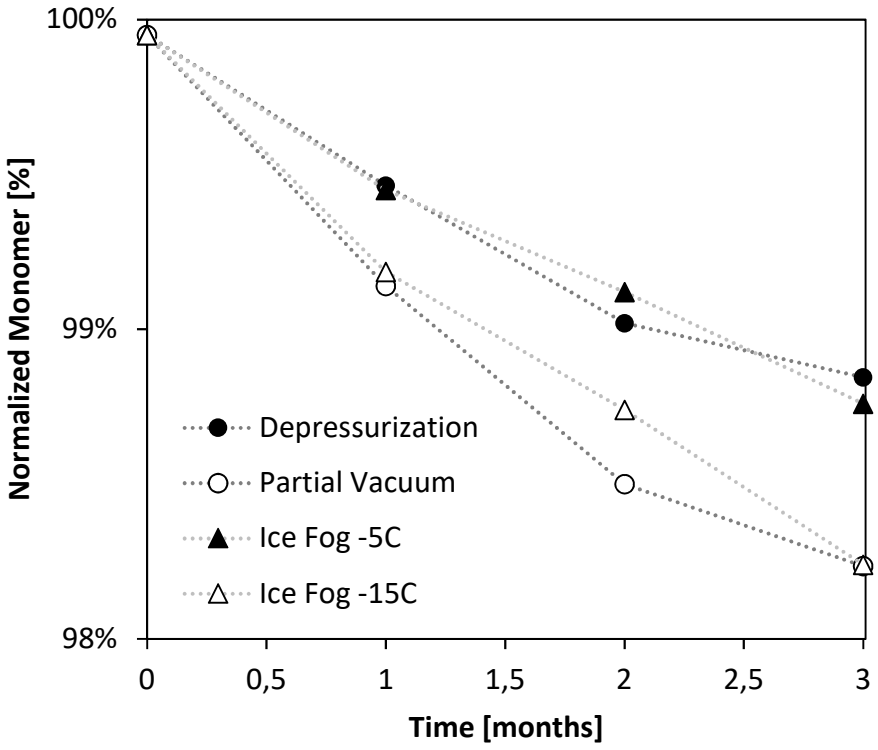


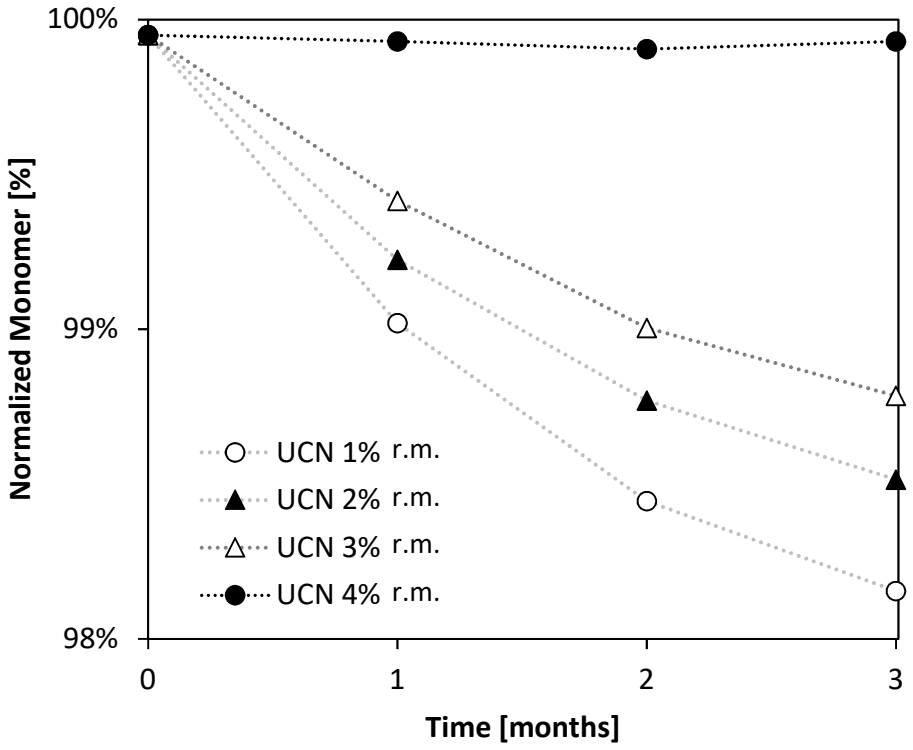
- Nucleation ten degrees apart resulted in large changes in cake structure and macroscopic cake structure

Nucleation at different temperatures

– stress stability (SEC, 40°C)

Formulation 2: 100 mg/mL mAb, nucleation temperature: -5° C and -15° C (20cc vial)





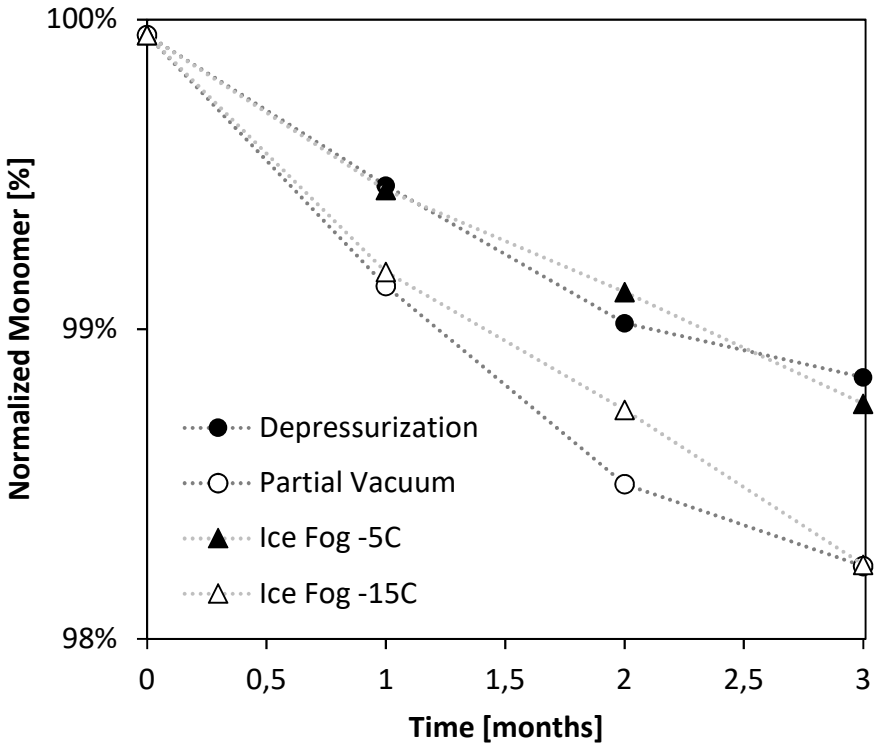
UCN = uncrontrolled nucleated, r.m. = residual moisture

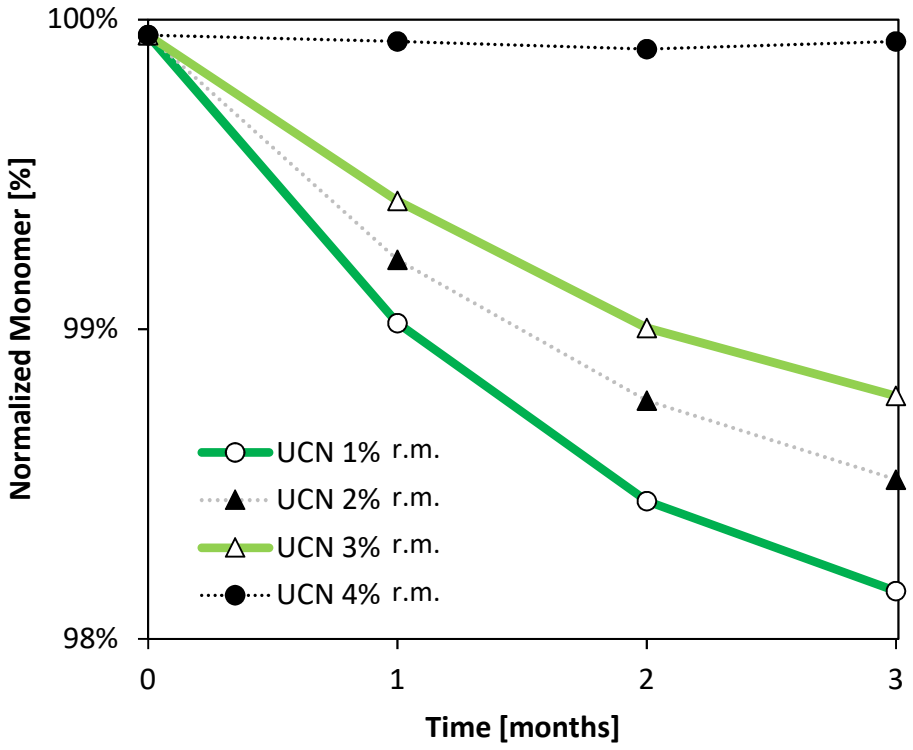
- Nucleation ten degrees apart resulted in different stability

Nucleation at different temperatures

– stress stability (SEC, 40°C)

Formulation 2: 100 mg/mL mAb, nucleation temperature: -5° C and -15° C (20cc vial)





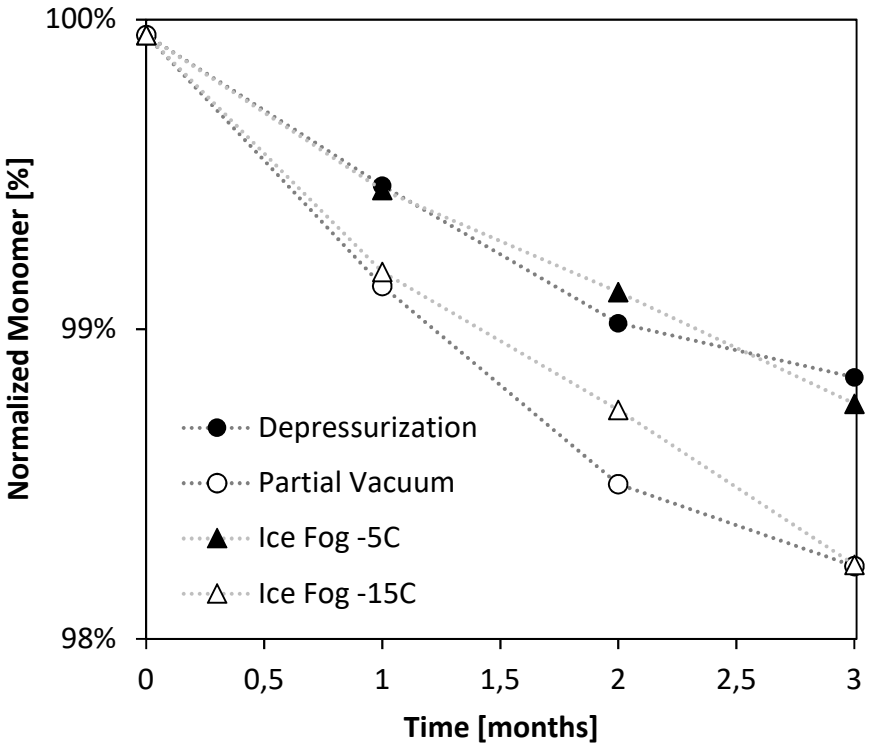
UCN = uncrontrolled nucleated, r.m. = residual moisture

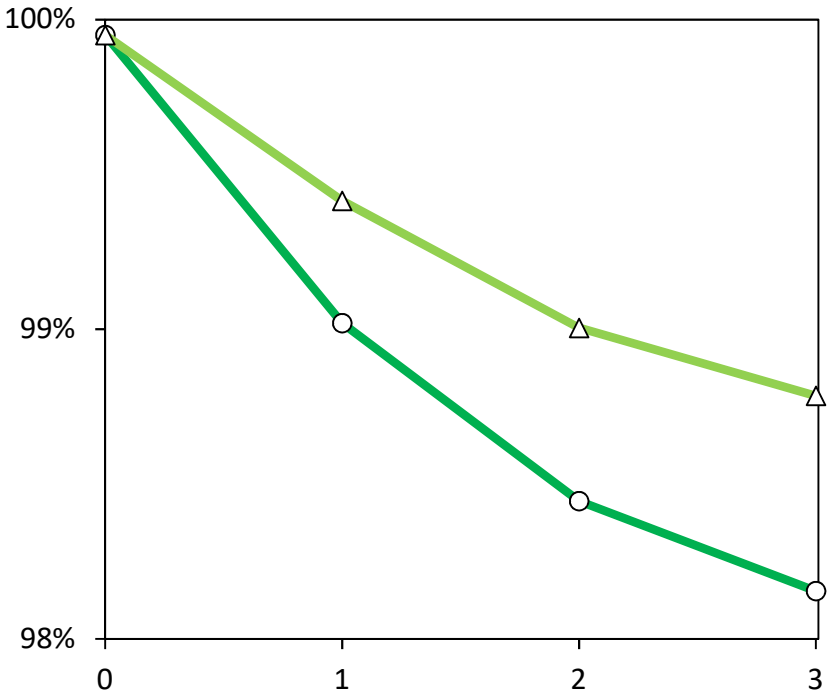
- Nucleation ten degrees apart resulted in different stability

Nucleation at different temperatures

– stress stability (SEC, 40°C)

Formulation 2: 100 mg/mL mAb, nucleation temperature: -5° C and -15° C (20cc vial)





- Nucleation ten degrees apart resulted in different stability

Nucleation temperatures achieved

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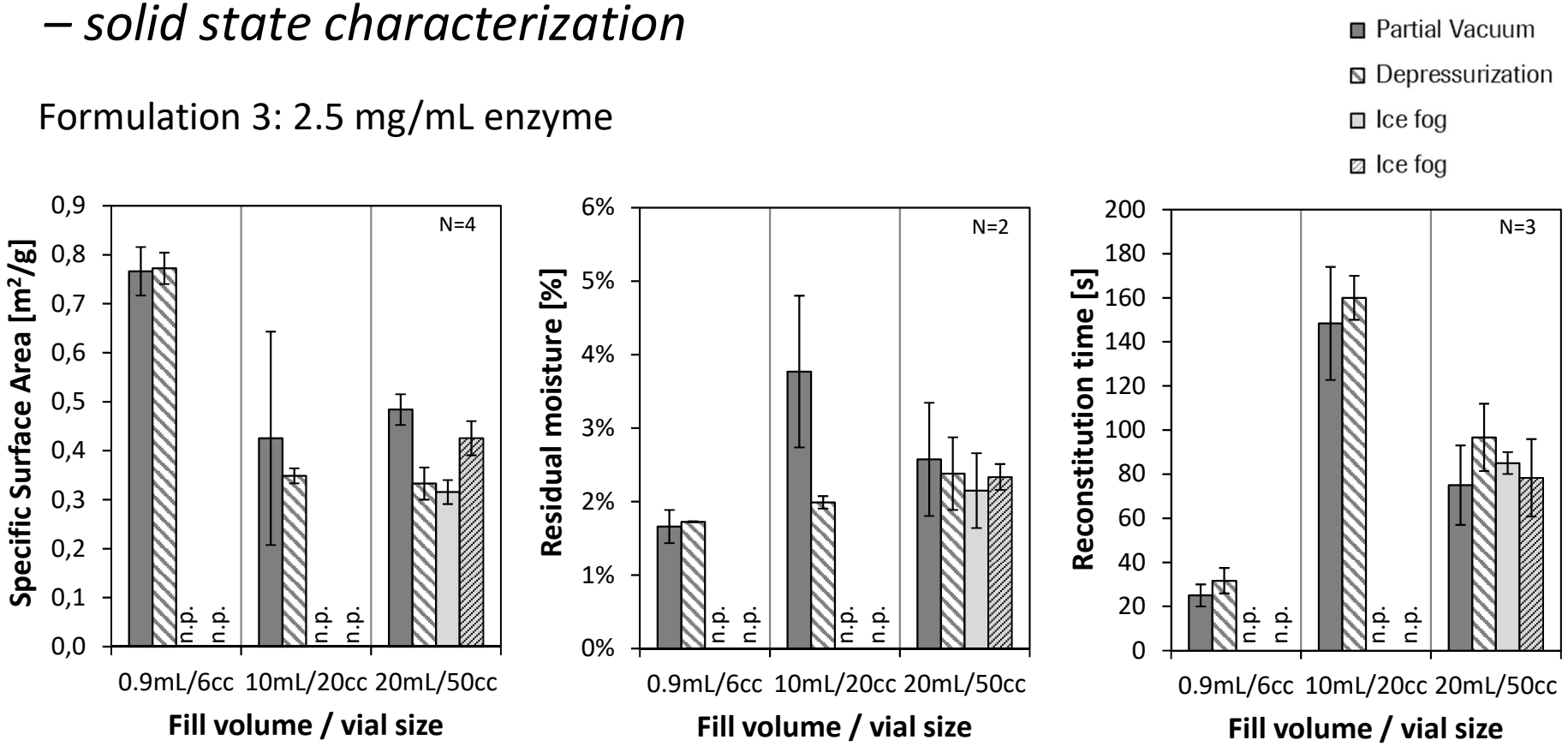
- Depressurization method struggled with 2cc vials
- Partial vacuum method struggled with Formulation 2/3 (high total solids)

Nucleation at different temperatures

– solid state characterization

Formulation 3: 2.5 mg/mL enzyme



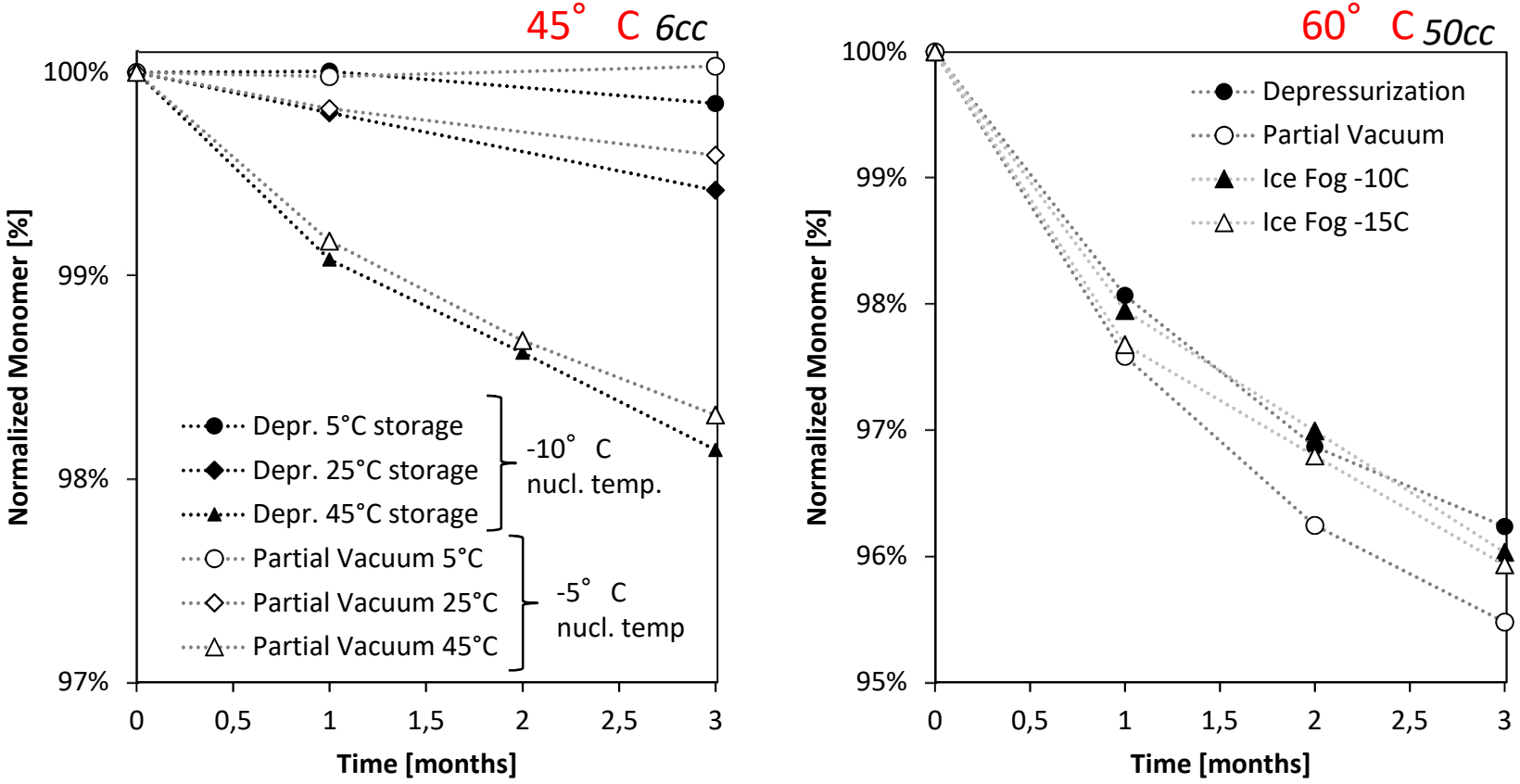
n.p. = not performed

- Nucleation five degrees apart resulted in in general comparable residual moisture and small changes to specific surface area

Nucleation at different temperatures

– stress stability (SEC°C)

Formulation 3: 2.5 mg/mL enzyme

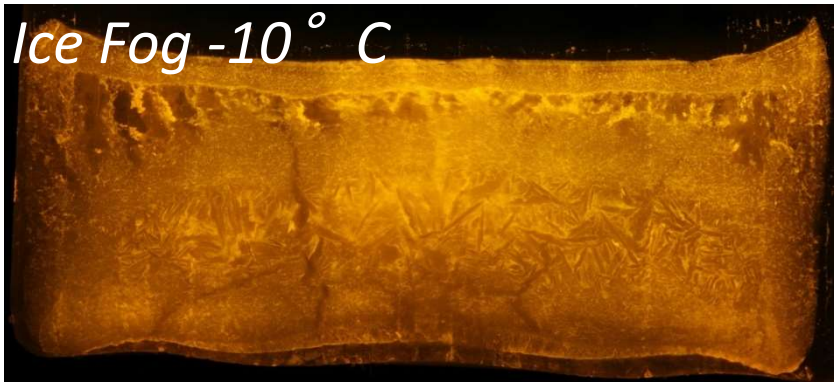


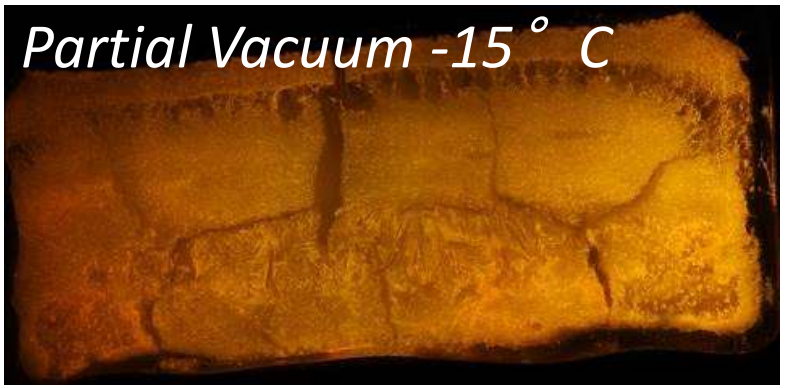
- Comparable solid state properties but different stability under stress conditions?

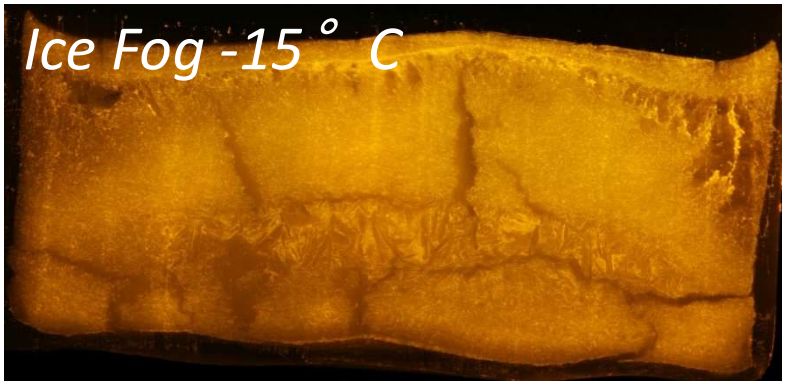
Nucleation at different temperatures

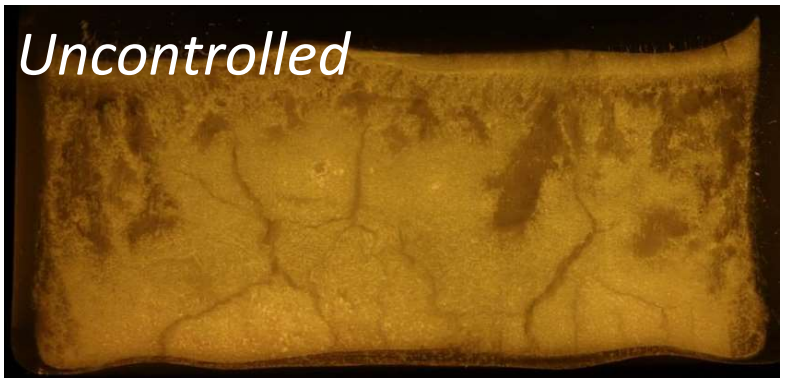
– macroscopic cake structure

Formulation 3: 2.5 mg/mL enzyme, 50cc









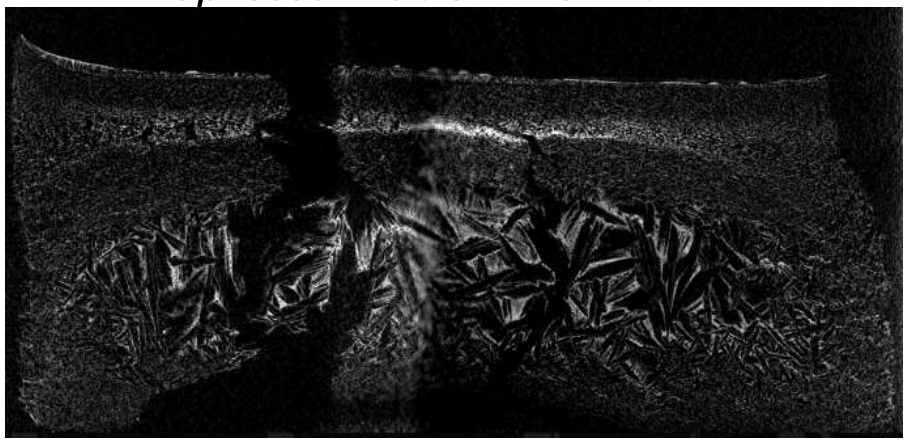
- Nucleation five degrees apart resulted in small changes to macroscopic cake structure

Nucleation at different temperatures

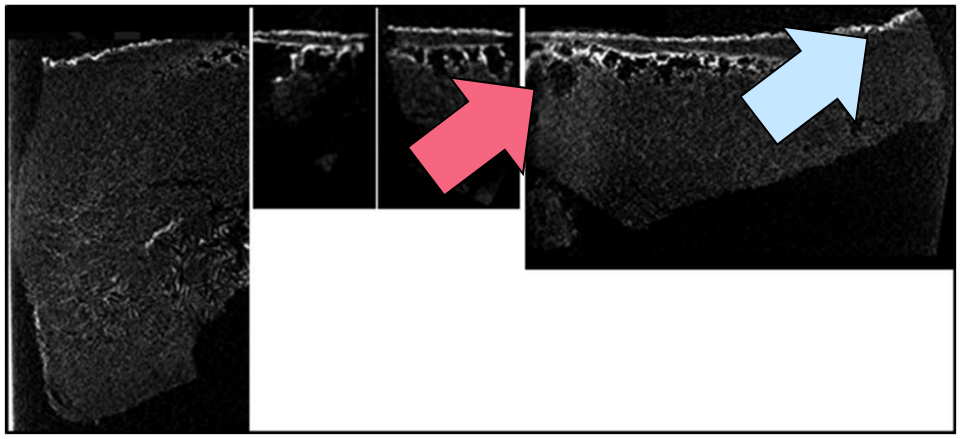
– macroscopic cake structure by μCT

Formulation 3: 2.5 mg/mL enzyme, 50cc

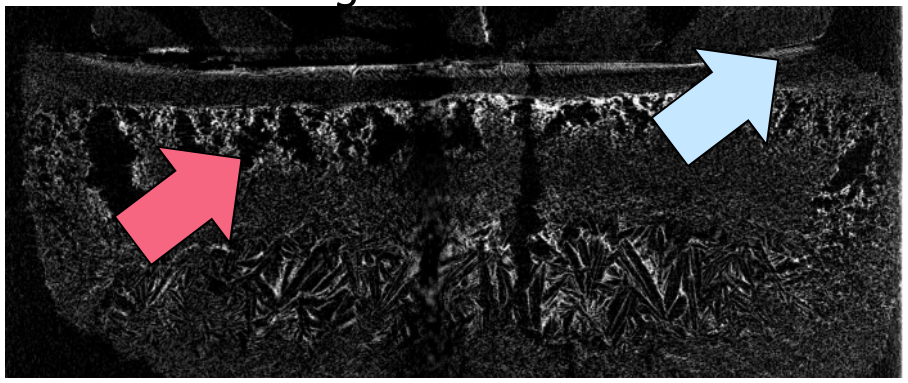
Depressurization -10° C



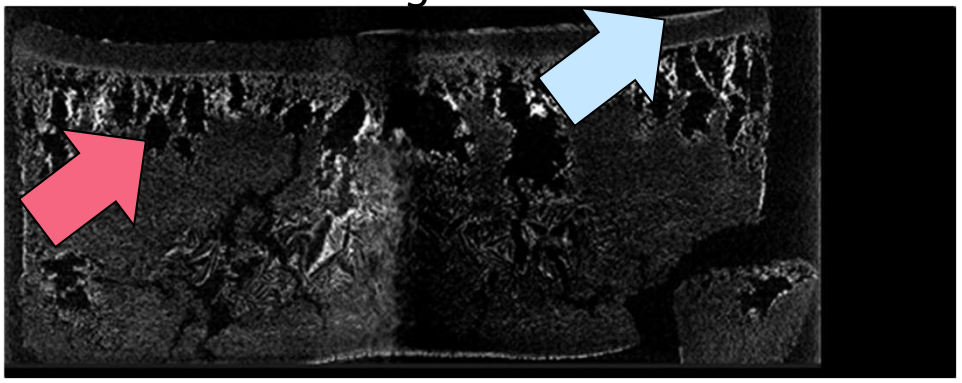
Partial Vacuum -15° C



Ice Fog -10° C



Ice Fog -15° C



- Differences in stability potentially due to microcollapse dependent on nucleation technique (enzyme is a surface sensitive molecule)?

Outline

Roche

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Background to controlling ice nucleation (CIN)

- Benefits
- Technologies

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Case-study:

Comparison of nucleation techniques

- Solid state
- Stability
- Challenges

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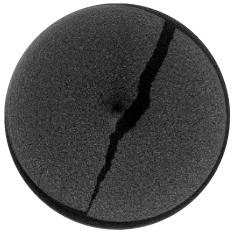
Summary and take-home messages

Summary

- Robustness testing for formulation and vial configuration revealed
  - Depressurization method struggled with 2cc vials
  - Partial vacuum method struggled with formulation with very high total solid content



- Nucleation at the same temperature resulted in comparable solid state properties like residual moisture and specific surface area, which directly relates to stability behavior dependent on the molecule studied

- Specific example showed that macroscopic structure (top layer) may be different between nucleation techniques, which may impact drying behavior, and is currently further studied

Take-home message

- Each technology has limitations
  - Depending on vial format and formulation you may need to nucleate at lower temperatures to ensure robust nucleation, which triggers formulation and configuration dependent process development
  - If operating conditions result in microcollapse, comparability between material produced with the different CIN technologies is not guaranteed
- Each technologies has different installation and operation requirements like availability, location and size of ports or availability of liquid nitrogen