Nucleation

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Volunteer for PDA

Overview

- Theory of Freezing
- Practical aspects of Freezing
- PAT
- R&D Equipment
- Recipe Parameters

Theory of Freezing

Nucleation

- Molecular energy is reduced (cooling)
- Formation of solid phase from liquid condition requires formation energy -- but releases more energy

⇒ phase change performs below freezing temperature when molecular energy level does not supply enough heat for de-nucleation

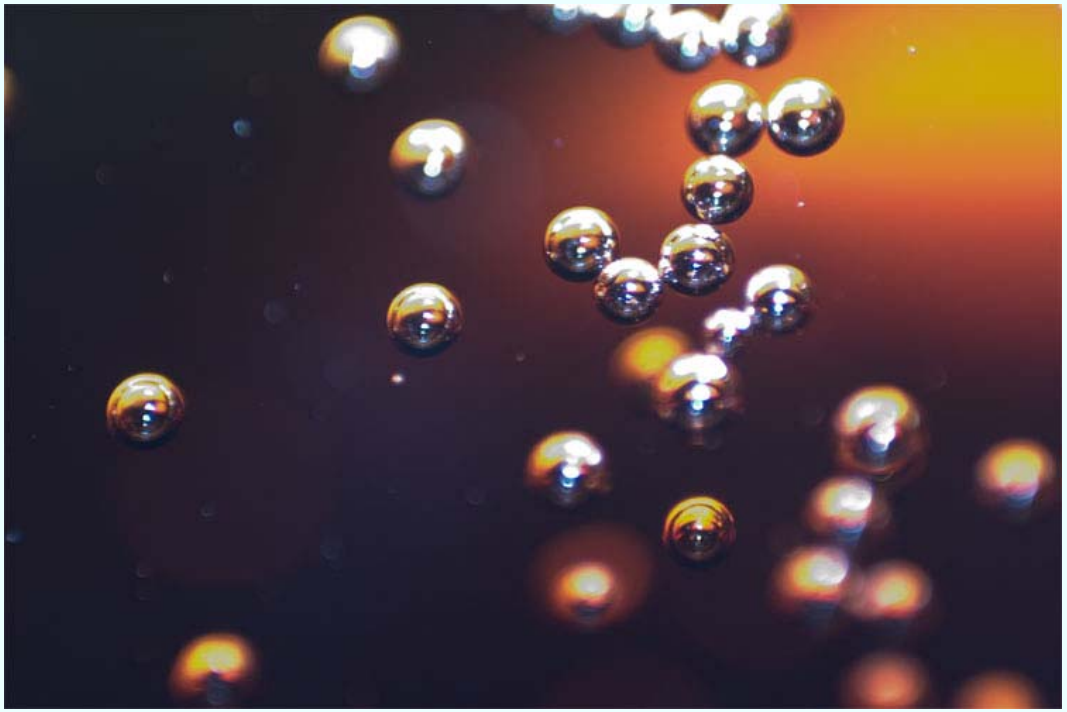
$$J = K \cdot e^{\frac{-\Delta G^*}{k_B \cdot T}}$$

[1]

Nucleation rate

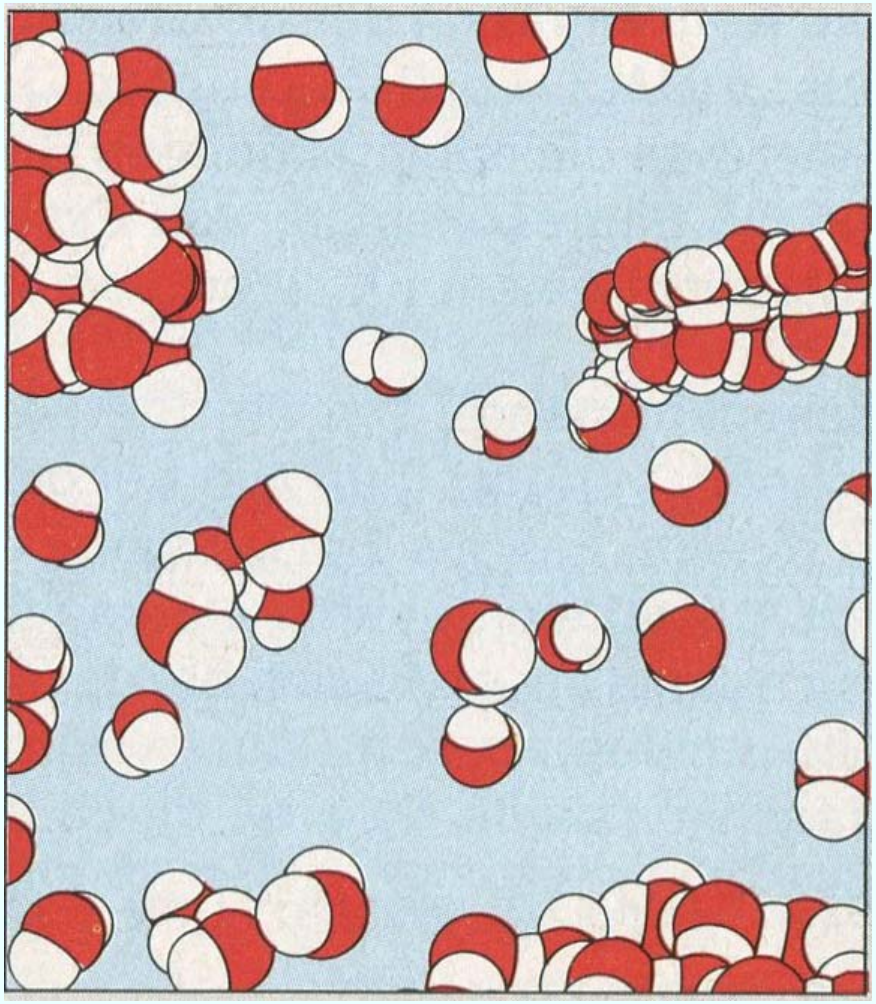
Nucleation

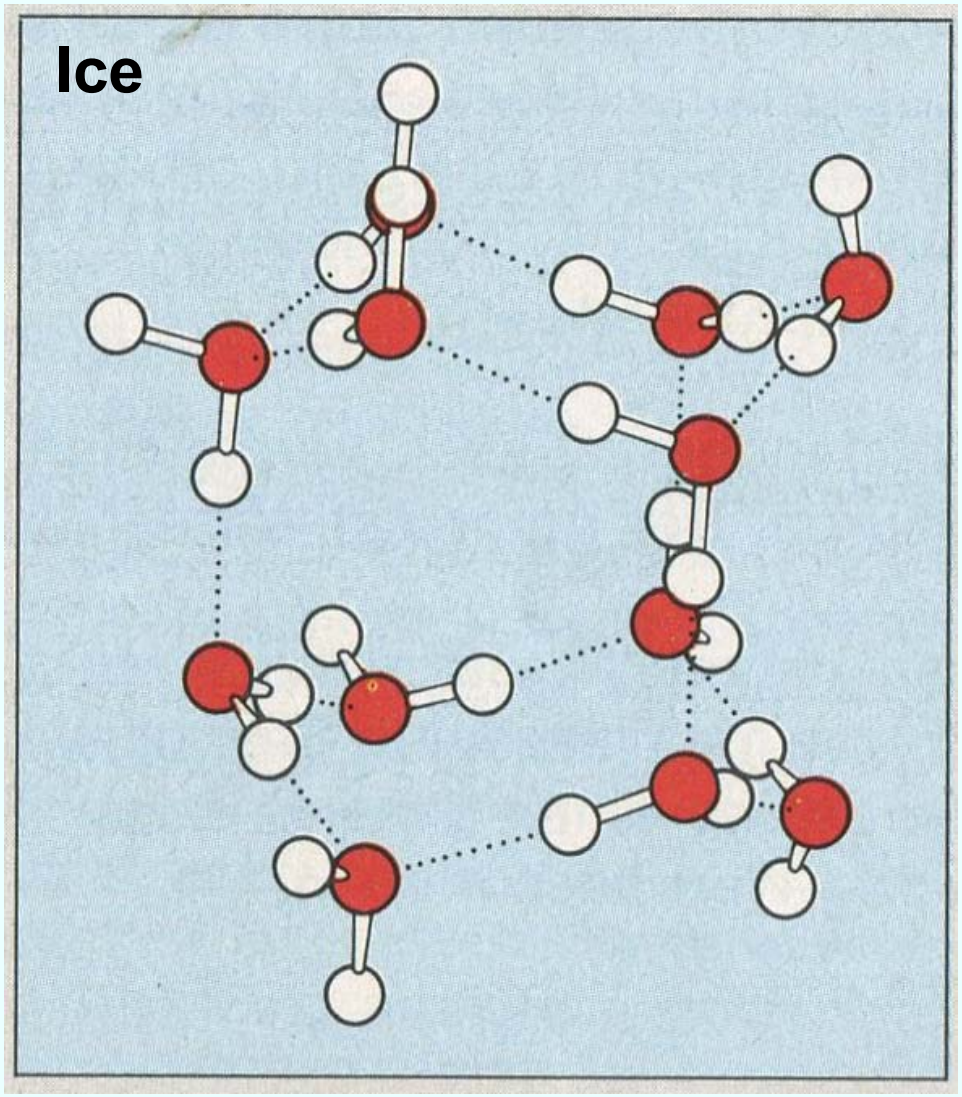
• The formation of bubbles in a cold, carbonated drink is also a good example for nucleation



Theory of Freezing

Cluster





Crystallization

Freezing of water lapses in several steps:

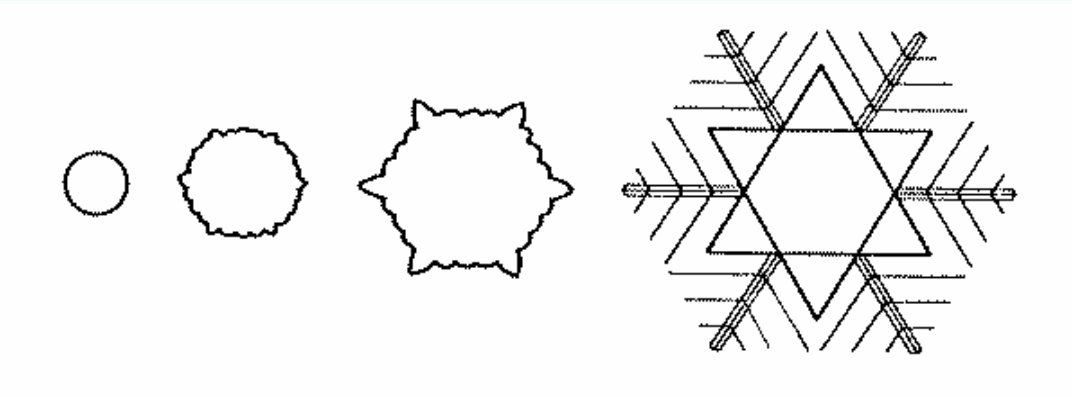
- First solid structure formed by Nucleus → Cluster → Ice crystals
- Homogenious Nucleation performs at _____ (guess!)
- After first nucleation liquid freezes immediately
- Freeze Drying of pure water makes no sense → Homogenious Nucleation is just an academic brainteaser

Crystallization

• Crystal growth depends on exposure time of liquid to seed crystals (Nuclei)

⇒ Long time for crystal growth allows compact crystals

⇒ short time (fast cooling) forms small and dendritic crystals



Ice crystals at different freezing speeds

Properties of Solution

- Freeze Drying is always based on Heterogenious Nucleation
- Nucleation starts around Ions, particels or amorphous material

Hailstone with core and growth rings

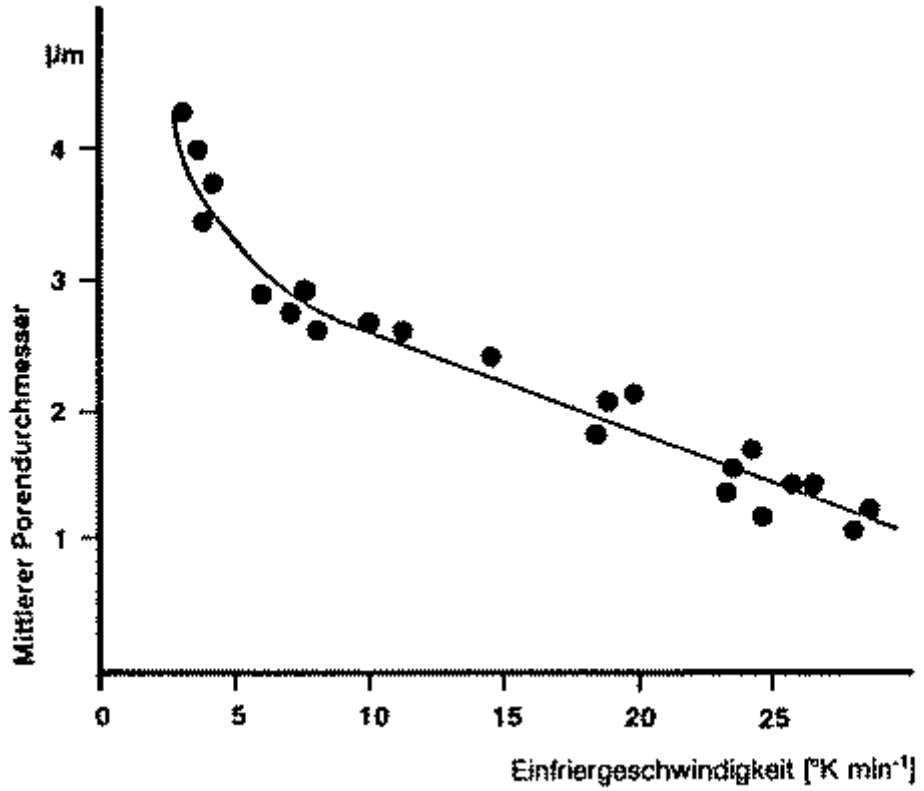
Crystallization

- Water solidifies always in dendritic structures
- Water never crystallizes in mixed crystals

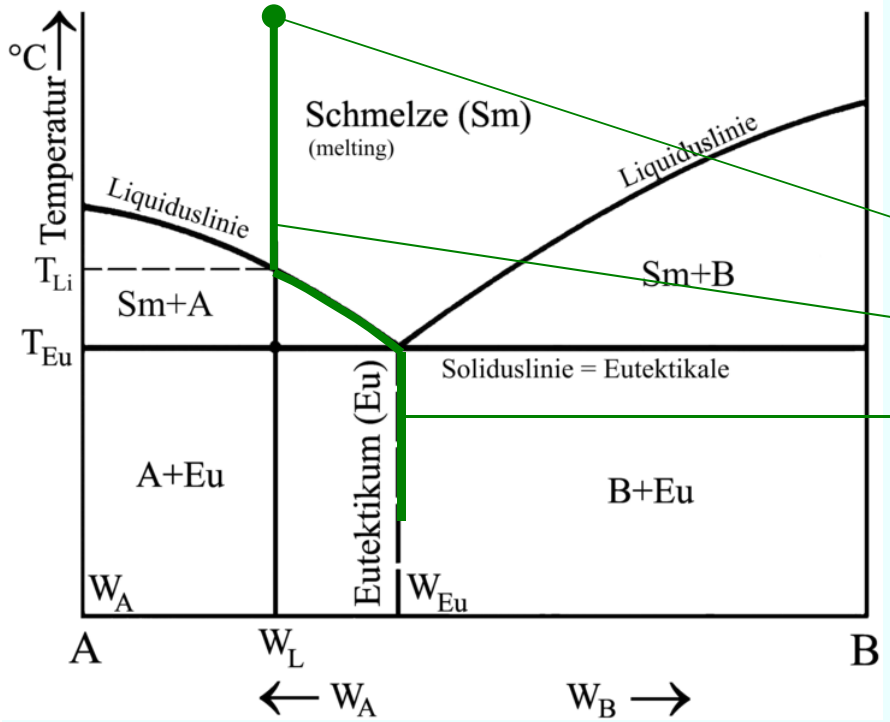
Frost pattern at window

Freezing of a solution

- Freezing rate impacts the crystal pore size reciprocally proportional
- The right freezing rate depends on product specific requirements
- 1K/min is not always the optimum freezing speed



Theory of Freezing



Principle of eutectic Freezing

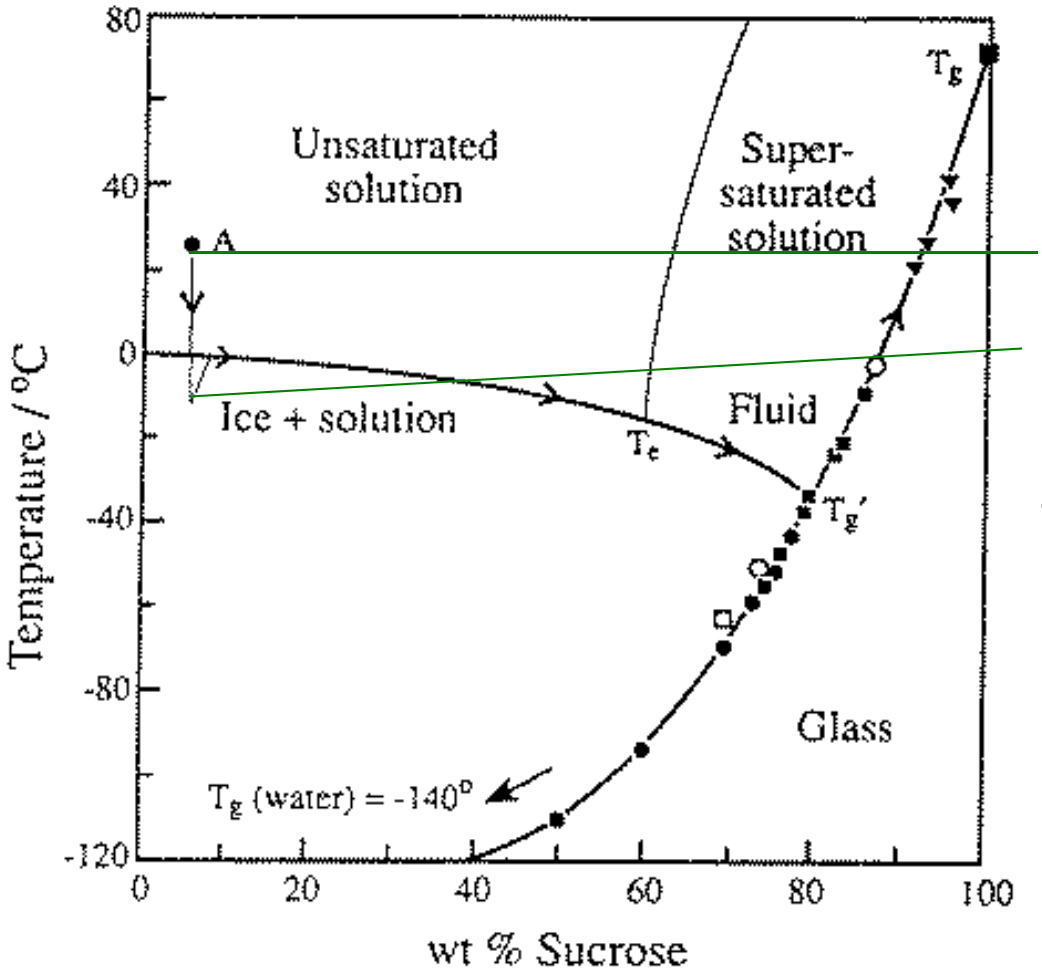
- Initial conditions
- Cooling before crystallization
- Subcooling depending on freezing rate
- Product solidifies in crystal structure
- Analogies to iron-carbon system exists - except mixed crystals

A: Solvent

B: Product

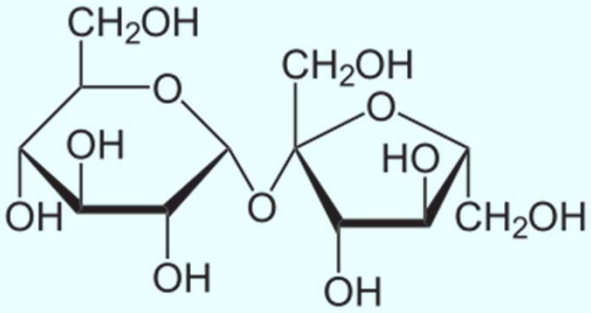
W: Concentration

Theory of Freezing

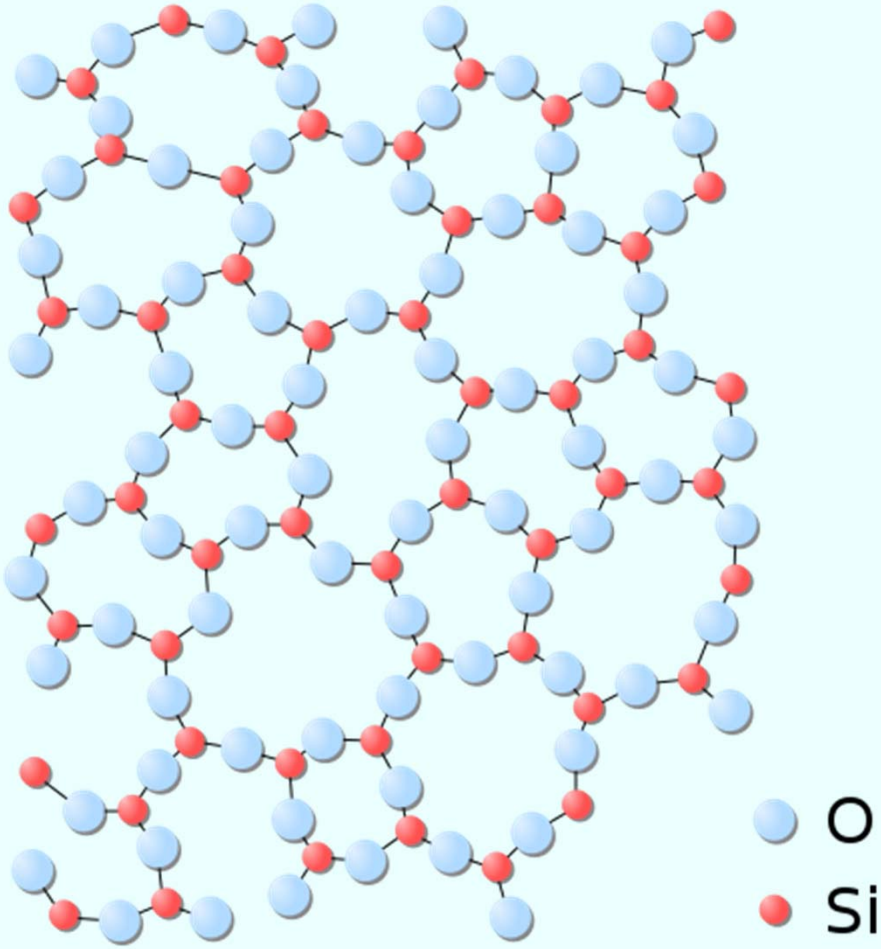


Principle of amorphous solidification

- Initial conditions
- Cooling before crystallization
- Subcooling depending on freezing rate
- When Product solidifies in amorphous (glassy) structure



Theory of Freezing



Molecular structure of glass

Amorphous (vitreous) structure

- no long-range-order for molecules
- Glass transition depends on water content
- Amporphous conditions are meta-stable
- Crystallization is exergonic => Devitrification means the process from forming a stable crystal structure
- Amorphous and crystallized material normally can not be distinguished with naked eye

Freezing of Formulations

- Depending on ingredients, there may be two solidification points (amorphous and crystalline), which need to be determined and evaluated.
- The result of this examination is the final freezing temperature, required for the specific formulation.
- When the product is solidified, the thawing/melting temperature becomes critical

  ⇒ Formulation specific *"Critical temperature": T_{crit}*

  ⇒ Overstepping of T_{crit} might cause Microcollaps or even complete collaps of the dried structure

  ⇒ "Controlled Nucleation uses initial nucleation of water (-5..-15°C)

Finally the liquid has safely solidified

Questions?

Practical aspects of Freezing

Freezing Phase

• There is no “standard” freezing rate of 1K/min, specific optimum to be investigated in advance

• The freezing ramp (silicone oil) should be safely below critical temperature to maintain sufficient heat flux between shelf and vial

• The shelf temperature must hold there to allow equalization of the temperature profile *(10min hold time per mm Layer height)*

Freezing Phase

• Proper freezing should be checked by previous freezing trials to verify sufficient freezing time (safety margin to the worst measured time)

• Ramp up should be performed as fast as possible with long hold times for equalization to reduce process variability

• Leading to next phase (sublimation) requires a cold ice condenser and process vacuum

Freezing Rate

Optimizing the freezing Rate has to consider some technical limitations

- The maximum achievable effective freezing rate (loading on pre-cooled shelves of a conventional Freeze Dryer) is below 5K/min (measured in the product)
- The effective freezing rate should be considered below 2K/min between 0...-40°C (high performing modern Unit)
- Higher requirements for the effective cooling rate must be fulfilled externally by bathing in refrigerant (e.g. liquid nitrogen)
- Silicone oil temperature, shelf surface temperature and product temperature are completely different parameters
- Freezing in R&D and Production units are different

Practical aspects of Freezing

General Equation for Heat Transfer

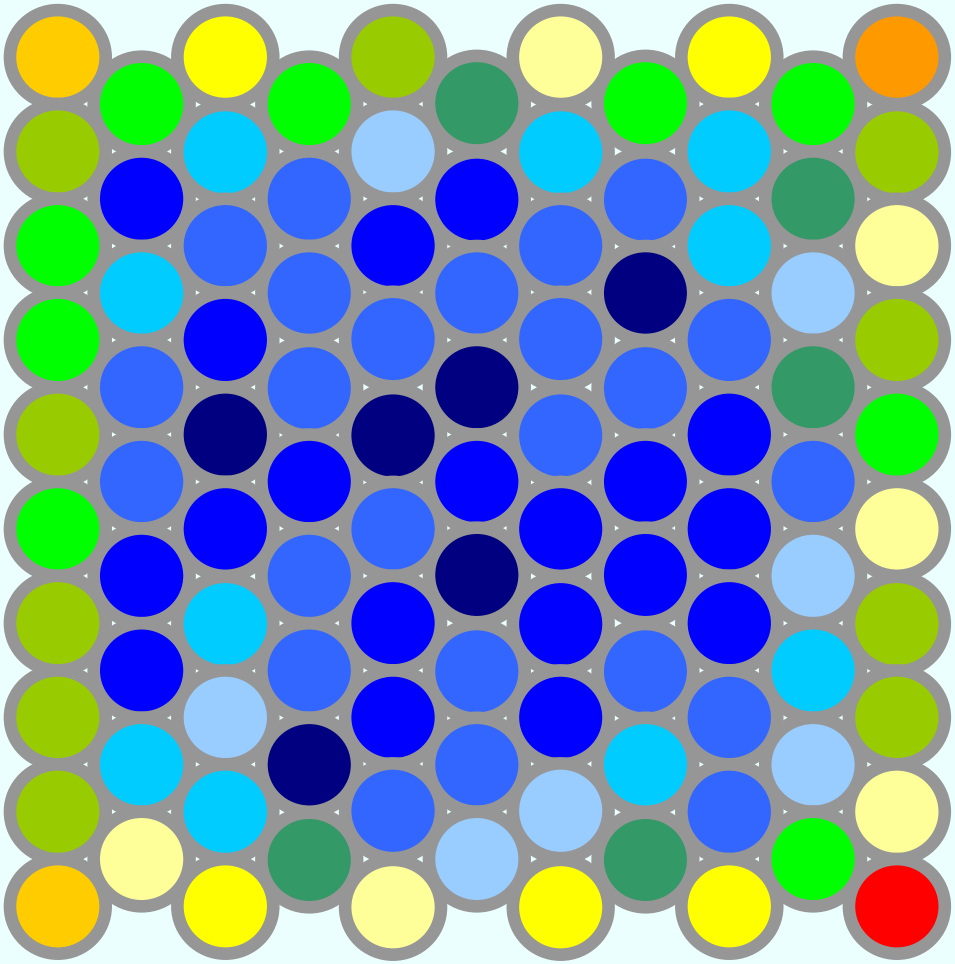
$$dQ_{Cond} + dQ_{Rad} + dQ_{Conv} = dQ_{comp}$$

$$\frac{dQ_{comp}}{dt} = k_V \cdot A_H \cdot [T_{Siliconeoil} - T_{Product}]$$

k_V	Very simplified Heat Transfer Coefficient
A_H	Vial Bottom Area
$T_{Product}$	Product at the Sublimation front

Impact of different Heat intake on Homogeneity

Vialtype:	10ml Vial
Filling:	5ml
Layer:	8mm
T_{Sh}:	0°C
T_{Rad}:	0°C
p_{ch}:	80µbar
t_{End}:	8h
m_{min}:	1,88g; 0,24g/h
m_{max}:	3,64g; 0,45g/h
Base:	3 runs aver.



	min	max
	1,85g	1,98g
	1,98g	2,10g
	2,10g	2,23g
	2,23g	2,35g
	2,35g	2,48g
	2,48g	2,60g
	2,60g	2,73g
	2,73g	2,85g
	2,85g	2,98g
	2,98g	3,10g
	3,10g	3,23g
	3,23g	3,35g
	3,35g	3,48g
	3,48g	5,00g

Freezing Rate

Some goods vs. evils have to be taken into consideration for recipe development

Slow Freezing	- reduces costs of production equipment - required for some cells (fungi, bacteria, viruses) - allows large pore size for better vapor flow during sublimation But: - Concentration change could cause pH-shifts - Proteins may denature - Cycle time and cycle variation increases
Quick freezing	- required for some cells or proteins - homogeneous frozen structure

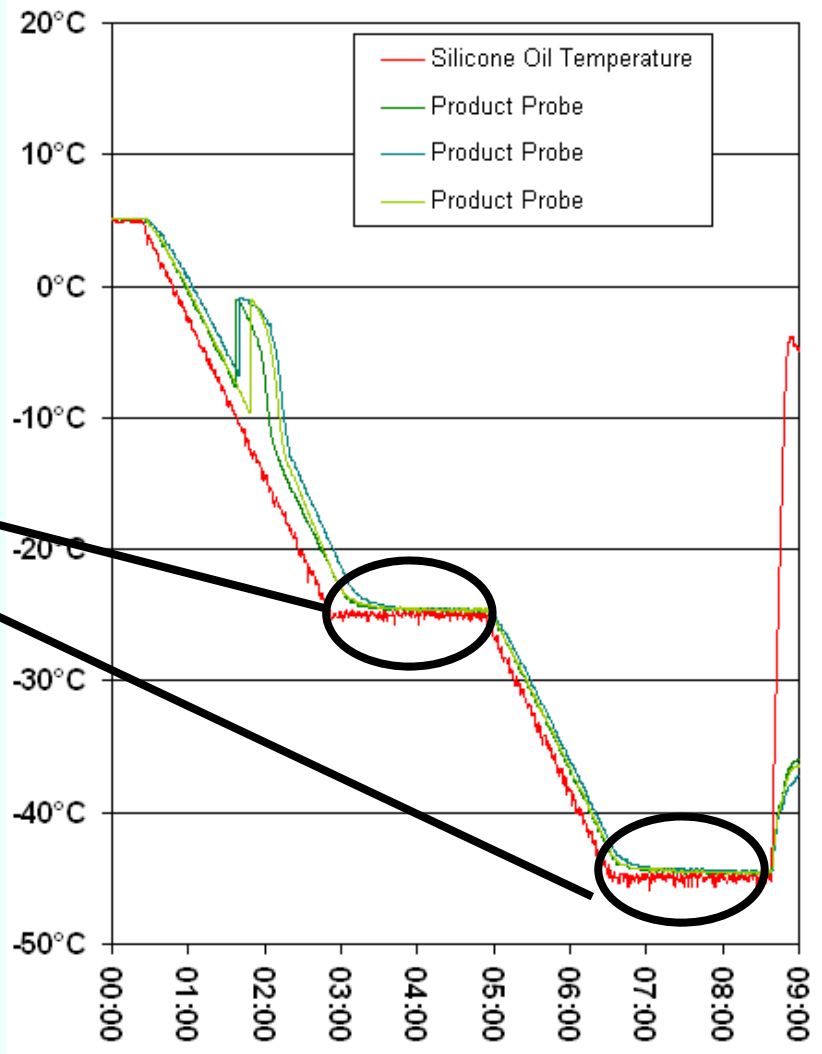
Practical aspects of Freezing

Freezing Phase

Estimation of freezing rate, freezing target temperature and hold time

Time Margins required

Final Freezing temperature should be min 15°C below critical temperature



Parameters to be determined for right Freezing

- Critical temperature of frozen product ($t_{Collapse}$ / t_{Glass} / $t_{Eutectic}$)

  ⇒ **We have to freeze safely below that temperature and wait there...**

  Reasons for an inhomogenious freezing profile at the batch:
  - *Edge Effect due to convecting air during freezing between cooling shelves and "warm" Chamber wall*
  - *Edge Effect due to dynamic thermal conditions at the shelf during freezing*
  - *Manufacturing tolerances of vials*
  - *Statistic Nucleation*

  ⇒ **Countermeasurements:**
  - *Hold time at cold temperature to wait for "slow" vials ("10Minutes per mm")*
  - *Thermal Treatment*

Thermal Treatment / Annealing

- Increase of temperature after freezing effects
  - (Re-)Crystallization
  - Structure Maturation

- A temperature range near the critical temperature should be selected
- Crystal pore size increases enabling reduced vapor flow resistance

⇒ **The advantage of reduced sublimation time has to be assessed with the time loss required for Thermal Treatment**

⇒ **Growth of ice crystals might destroy the membranes of living cells or denature proteins**

PAT

„True PAT“ ≡ *Three Laws*

1. Pharmaceutical Compliance (full GMP)
   - Aseptic design
   - Sterile Conditions
   - Certified Materials & Manufacturing
   - Secured Process Control (GAMP)

„True PAT“ ≡ *Three Laws*

2. Process Feedback (inline and online)
   - Full Batch
   - No Process Interference/Disturbance

3. Reaction on Process Feedback possible (inline and online)

=> Most available “so-called” PAT Tools are just development tools.

PAT @ Freezing:

Process Control for Nucleation

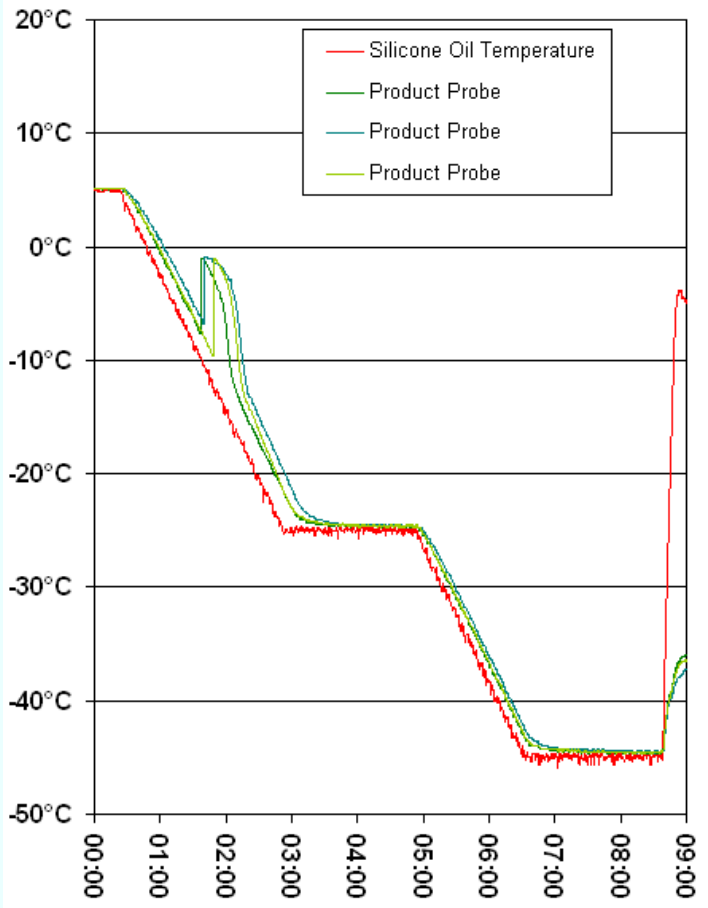
- Mechanical Events like (Super) Sonic, Pressure Waves, Shelf Vibrations, Shelf Motion, initiate instant Nucleation

Process Feedback for Nucleation

- NIR
- X-Ray (Diffraction)
- Micro Wave Absorption (?)
- Impedance Spectra (?)
- Sonic Resonance (?)

PAT @ Freezing:

- Time margins required anytime
- Increased Homogeneity of Nucleation when triggered
- Controlled Nucleation with Process Feedback thinkable



R&D Equipment

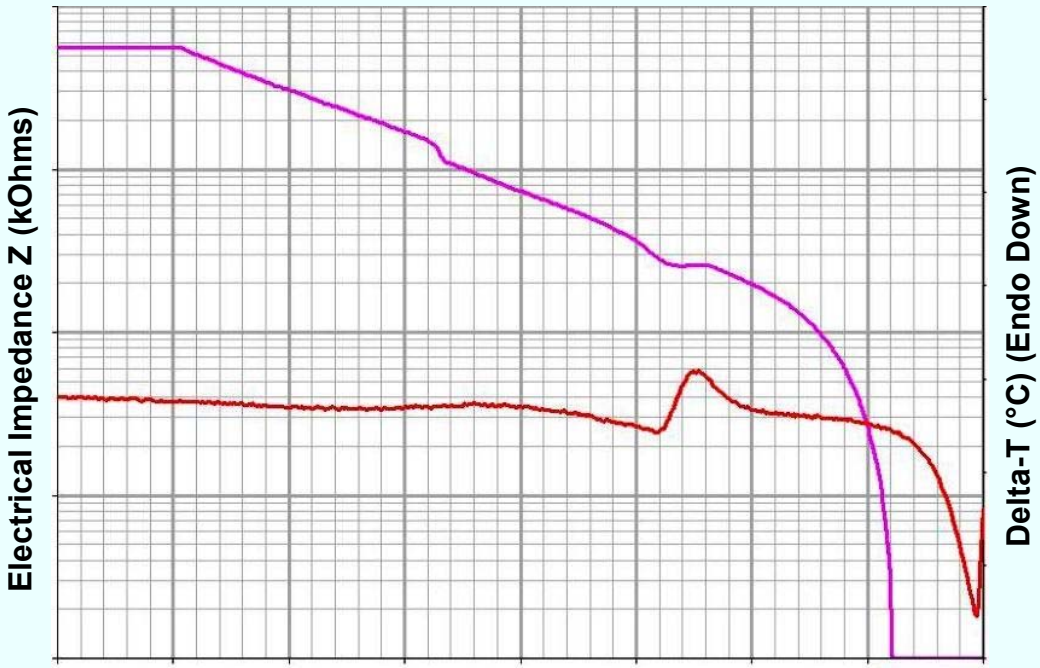
Research Equipment for Freezing Phase

- Temperature – Resistance measurement
- Differential Scanning Calorimetry
- Freeze Drying Microscope

“Mistakes done at freezing phase have an tremendous (terrible) impact on the further drying process”

Temperature Resistance Measurement

- Electrical Resistance (Impedance) and Probe Temperature is measured and recorded
- Resistance is proportional with temperature
- Non-linear change of Resistance indicates a change of molecular structure, especially a phase change



[1]

Temperature Resistance Measurement

- Measurement Amplifier & Control Unit
- Heating & Cooling System

[1]

Temperature Resistance Measurement enables:

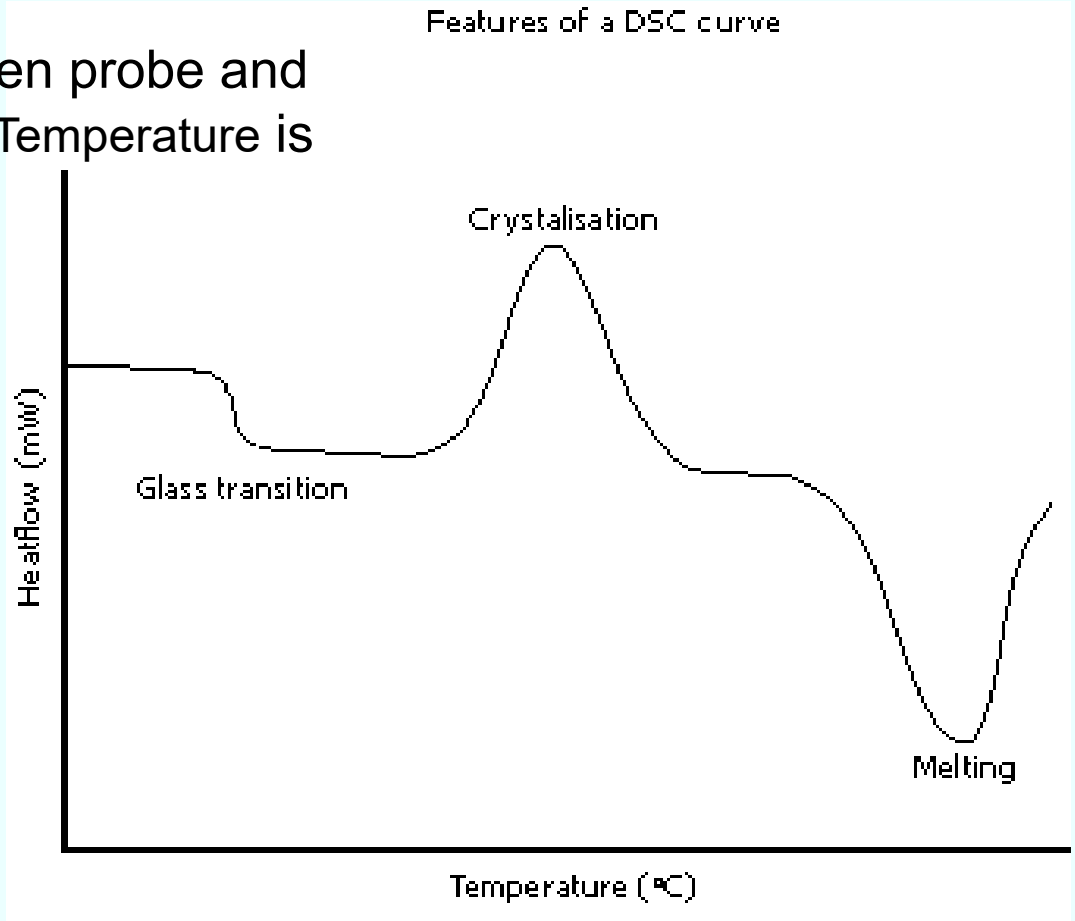
- Detection of Solidifying/Melting Points
- Investigation of Relation between Freezing Rate and t_{crit}
- Simulation of Thermal Treatment and monitoring its effects

...requires:

- 2 hours of preparation & analyzing time for a well experienced person
- 15.000...20.000€ as Capital Expense
- Availability of small amounts of Liquid Nitrogen (< 5kg)

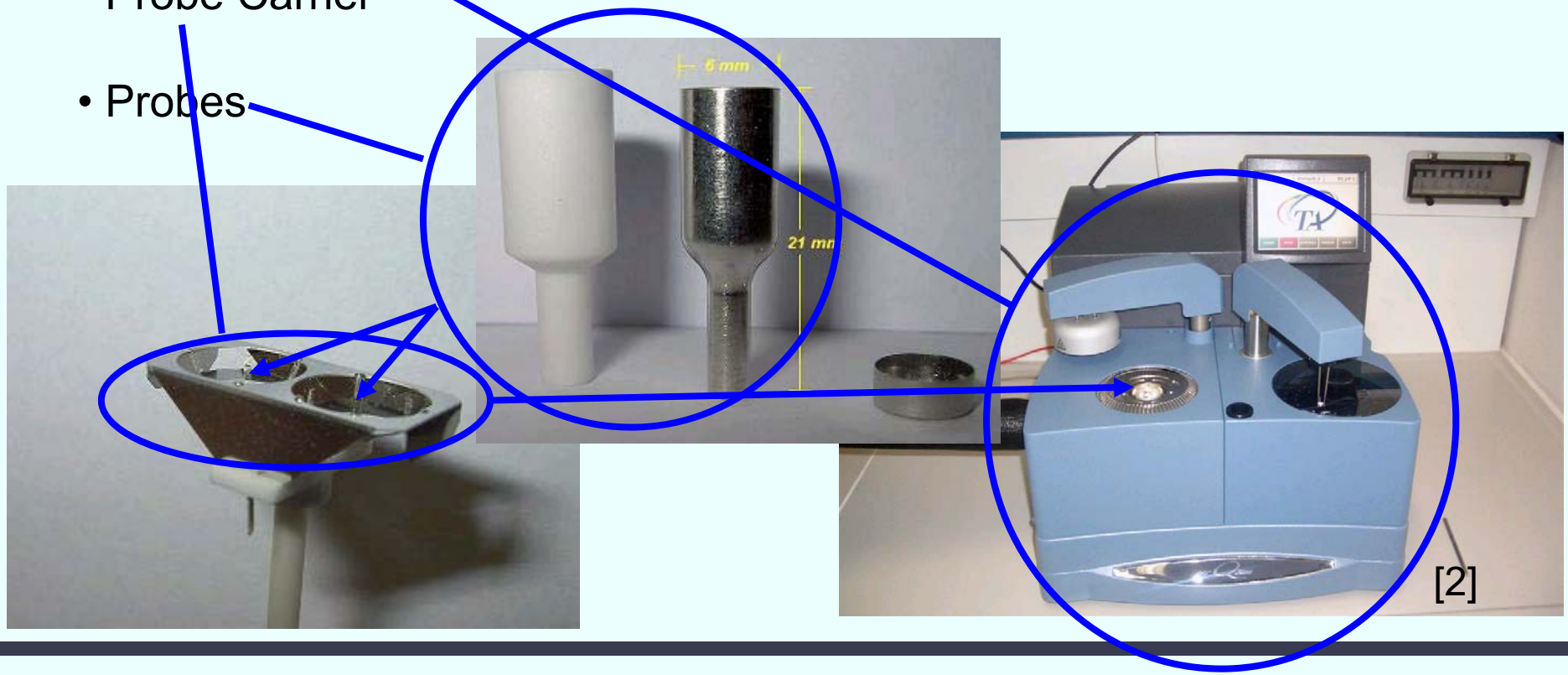
Differential Scanning Calorimetry

- Modifying Heat Flow between probe and empty references and Probe Temperature is measured and recorded
- Heat Capacity changes with phase modification
- Endothermic/Exothermic Reactions can be investigated and monitored



Differential Scanning Calorimetry

- System
- Probe Carrier
- Probes



[2]

Differential Scanning Calorimetry enables:

- Detection of Solidifying/Melting Points
- Detection Glass Transition Points
- Investigation of Relation between Freezing Rate and T_{crit}
- Simulation of Thermal Treatment and monitoring of its effects

...requires:

- 25.000...40.000€ as Capital Expense

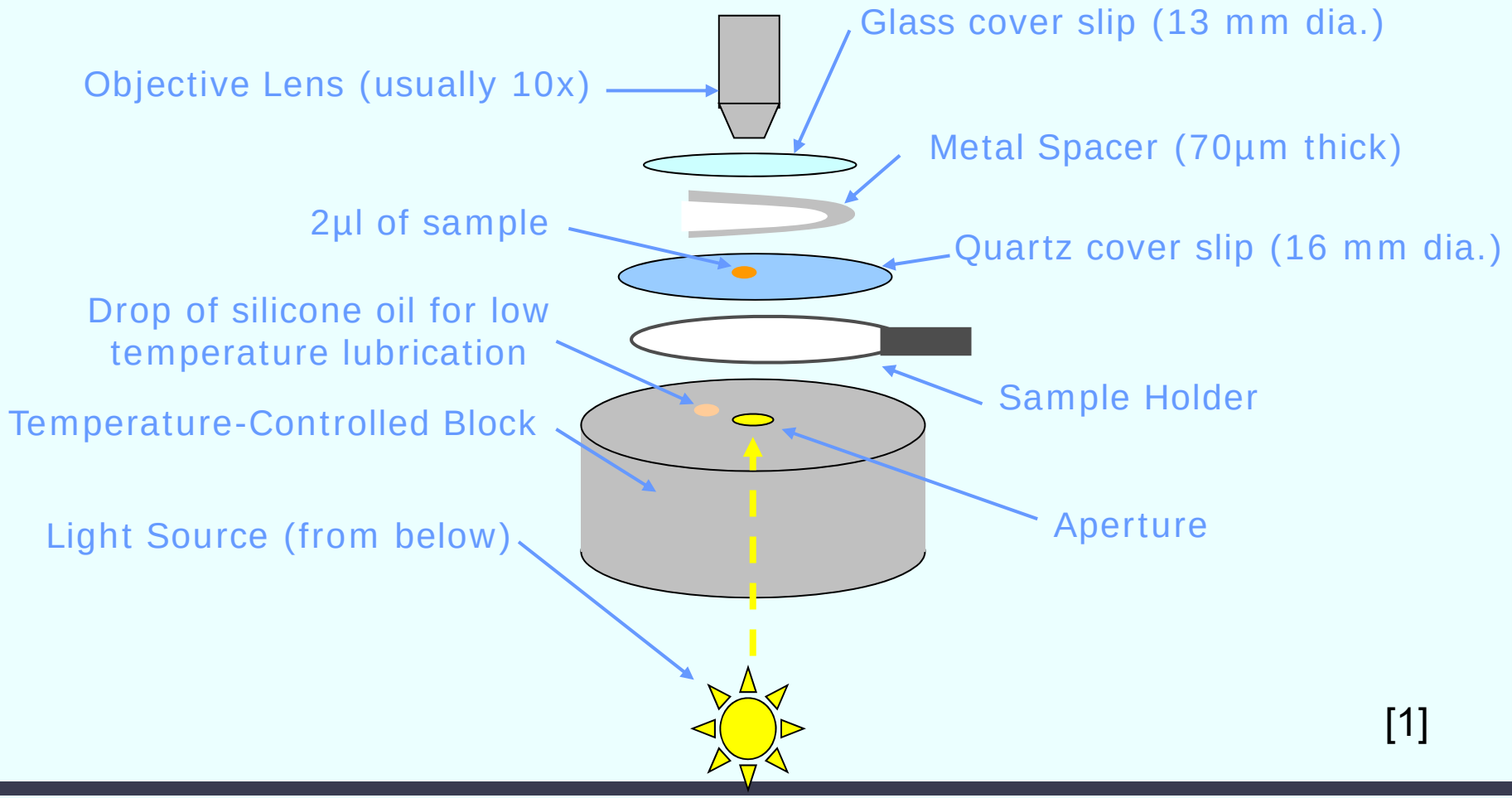
Freeze Drying Microscope

[2]

Freeze Drying Microscope

- Performing a real freeze drying process with online observation of freezing and sublimation phase
- Temperature controllable sample holder
- Vacuum controllable sample holder

Freeze Drying Microscope



[1]

Freeze Drying Microscope enables:

- Detection of Solidifying/Melting Points by direct observation
- Detection of Microcollapse and Collapse by direct observation
- Investigation of Relation between Freezing Rate and t_{crit}
- Investigation of Relation between Freezing Rate and structure
- Direct observation of Sublimation process
- Direct design of optimum Thermal Treatment and monitoring its effects

R&D Equipment

...requires:

- 1 hour of preparation time for a very well experienced person + the time for the process (might be compressed by a video system)
- 25.000...40.000€ as Capital Expense
- A vacuum control system (Pump, flow controller, Gauge)
- Availability of small amounts of Liquid Nitrogen (< 5kg)
- Data Mining Equipment for movie storage

R&D Equipment

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Thank you for your attention!

Questions?

Some Questions of mine 1:

- ✓ What is a cluster?
- ✓ Sort right Order (a Cluster; b Nucleus; c Ice Crystal):
- ✓ Heterogenious Nucleation?
- ✓ Eutectic Freezing?
- ✓ T_g‘?
- ✓ Relevant feature of amorphous?

Some Questions of mine 1:

✓ Hold time per mm of Layer?

✓ Required parameter for „right freezing“?

✓ Describe the characteristic of the Resistance function, while freezing:

✓ What means „true PAT“?

[1] Nucleation

In this example: gazous to liquid

$$\Delta G^{*} = \frac{16\pi}{3} \cdot \frac{v_l^2 \cdot \sigma^3}{(k_B \cdot T \cdot \ln(S))^2}$$

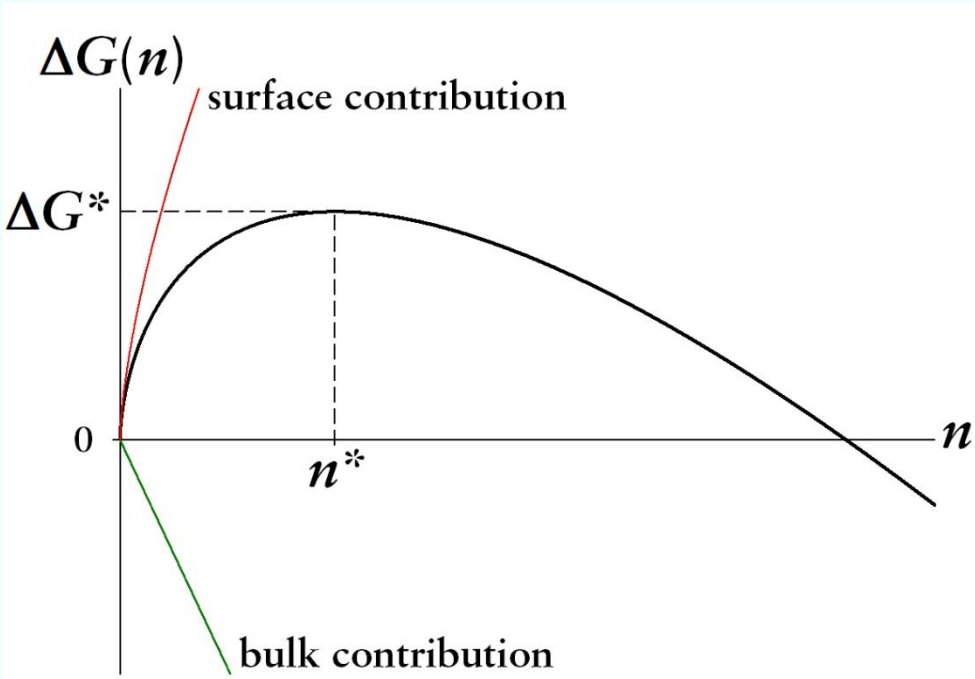
v average volume of molecule in liquid phase

σ surface tension of liquid

k_B Boltzmann constant

T absolute Temperature

S Entropy



Recommended lectures

- Felix Franks – *Water - The matrix of life*
- Georg Wilhelm Oetjen, Peter Haseley – *Freeze Drying (2nd. Edition)*
- Louis Rey, Joan C. May – *Freeze Drying of pharmaceutical products*