Theory 2

PDA EU
Freeze – Drying In Practice

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Martin Christ
Osterode am Harz, Germany

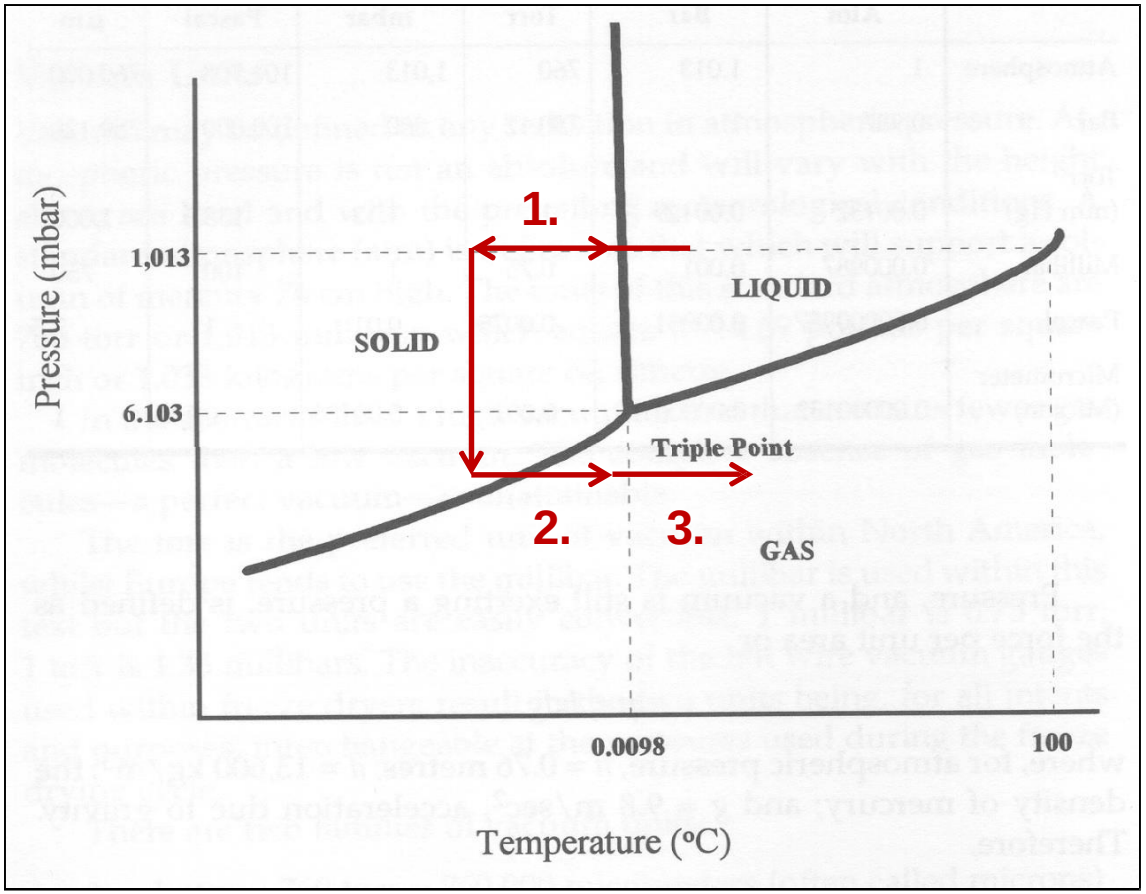
Adapted from slides originally created and kindly provided by PD Dr. Andrea Allmendinger

Theory 2

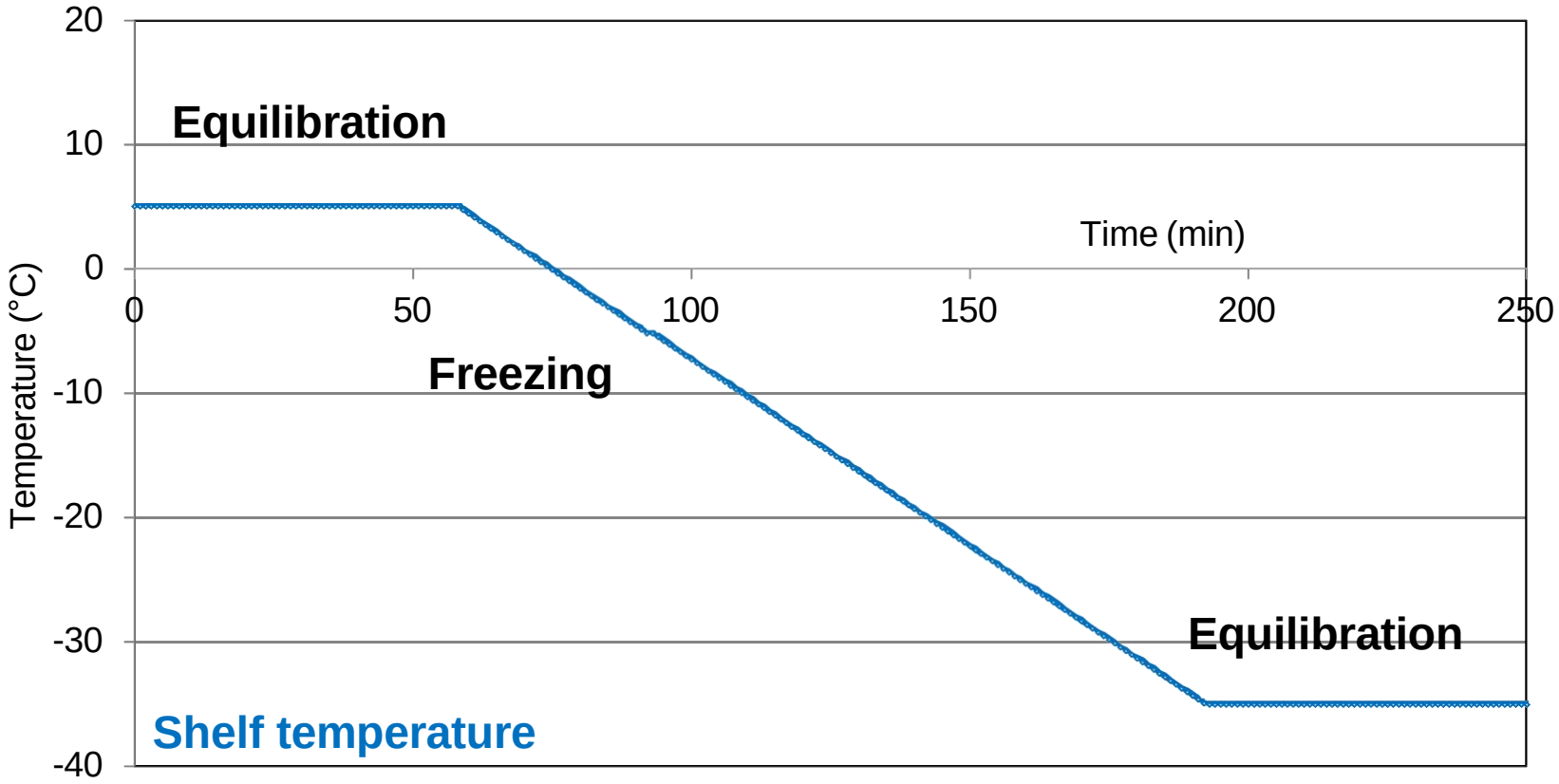
- Basic principles of freeze drying processes
  - Physical understanding
  - Critical process parameters
- Primary packaging components
- Development and composition of a (biological) formulation
- Analytical characterization:
  - Product attributes for designing lyophilization cycles
  - Solid state characterization after lyophilization

Basic principles

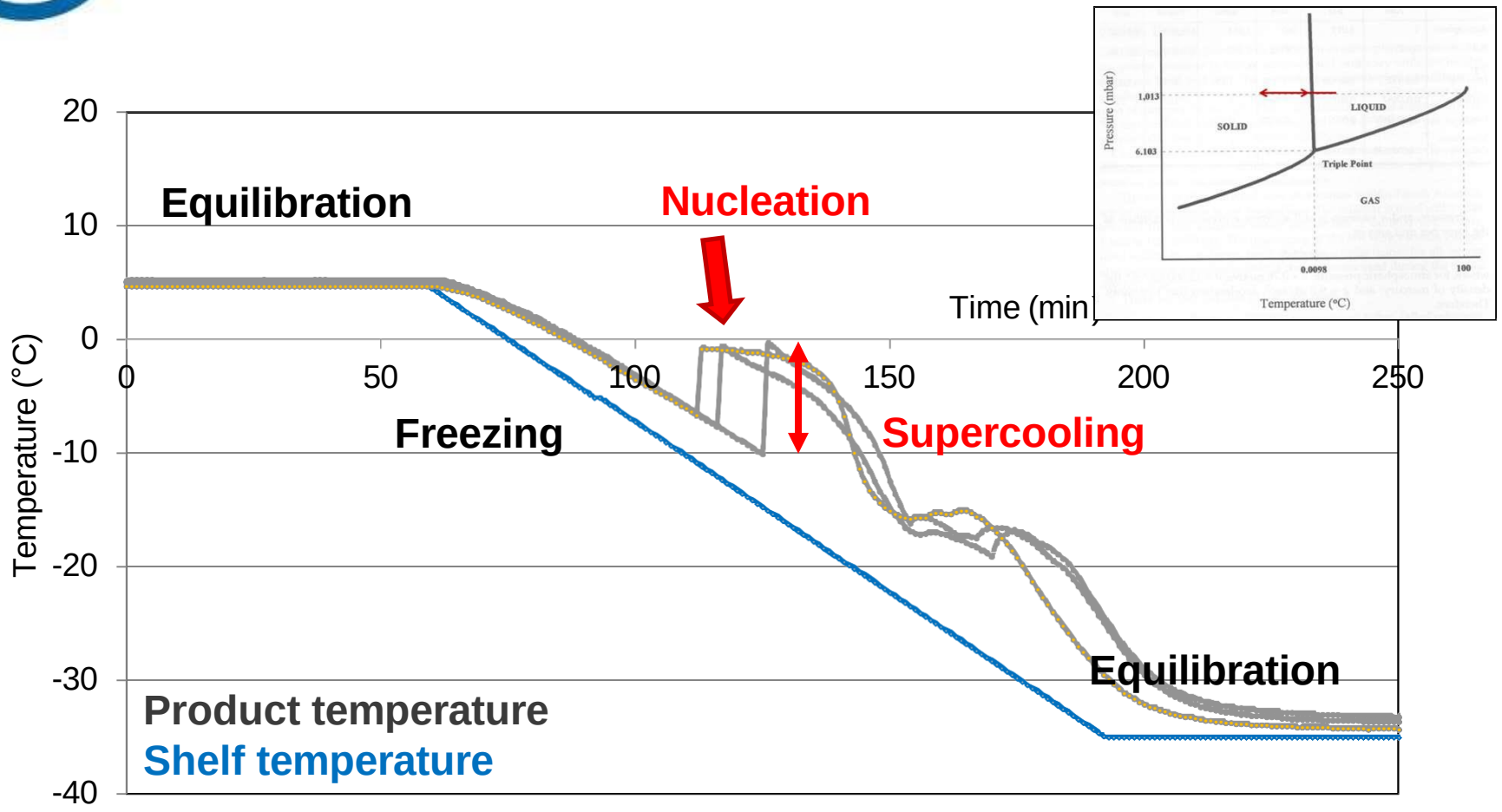
- Drying by sublimation of ice as well as desorption of adsorbed water
- **Phases**:
  - **1. Freezing phase**
    - approx. 2-10 h
  - **2. Primary drying**
    - approx. 5 h - 5 d
  - **3. Secondary drying**
    - < ~13h



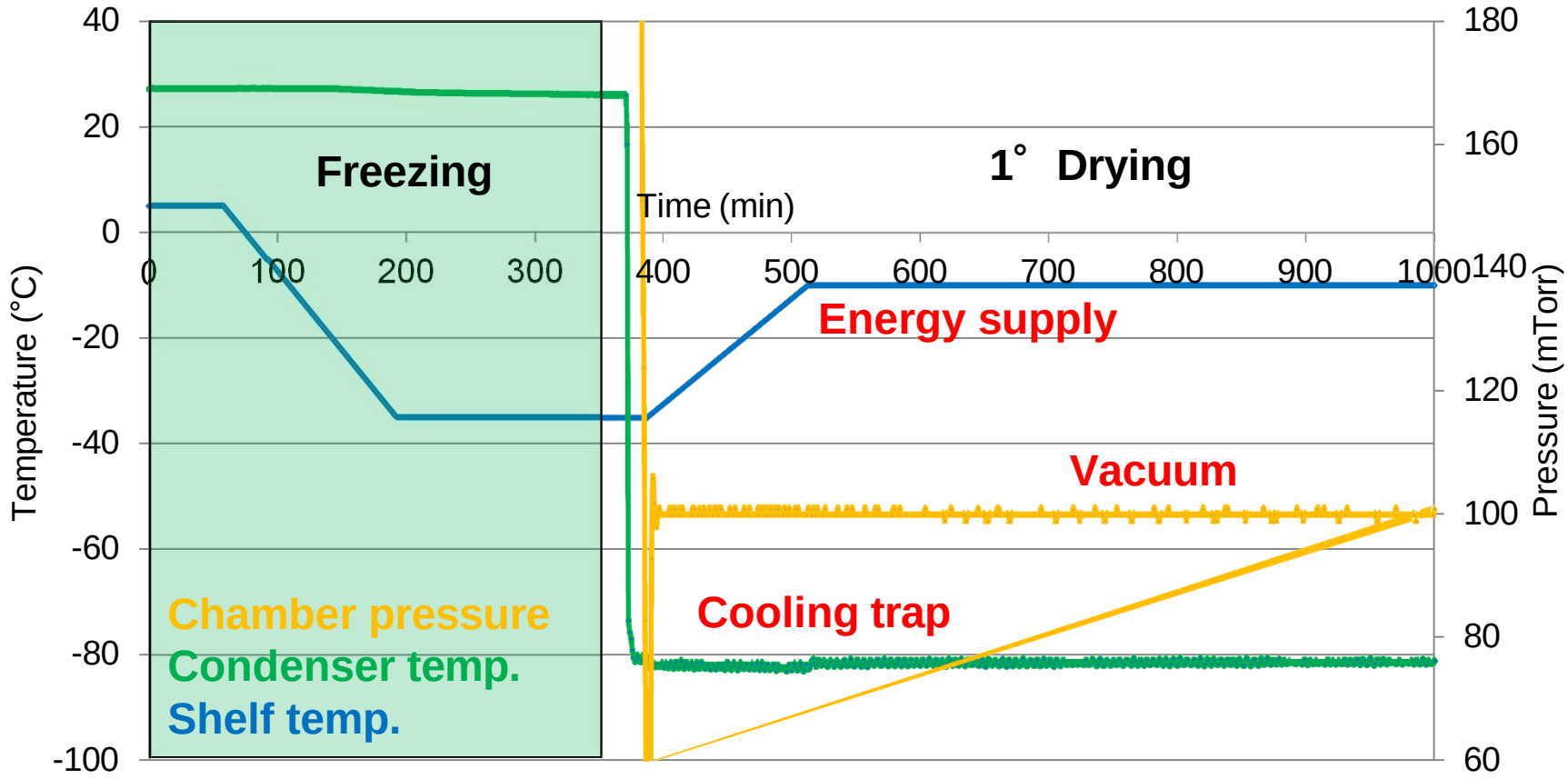
Freezing


20
10
0
-10
-20
-30
-40
Temperature (°C)
0
50
100
150
200
250
Time (min)
Equilibration
Freezing
Equilibration
Shelf temperature

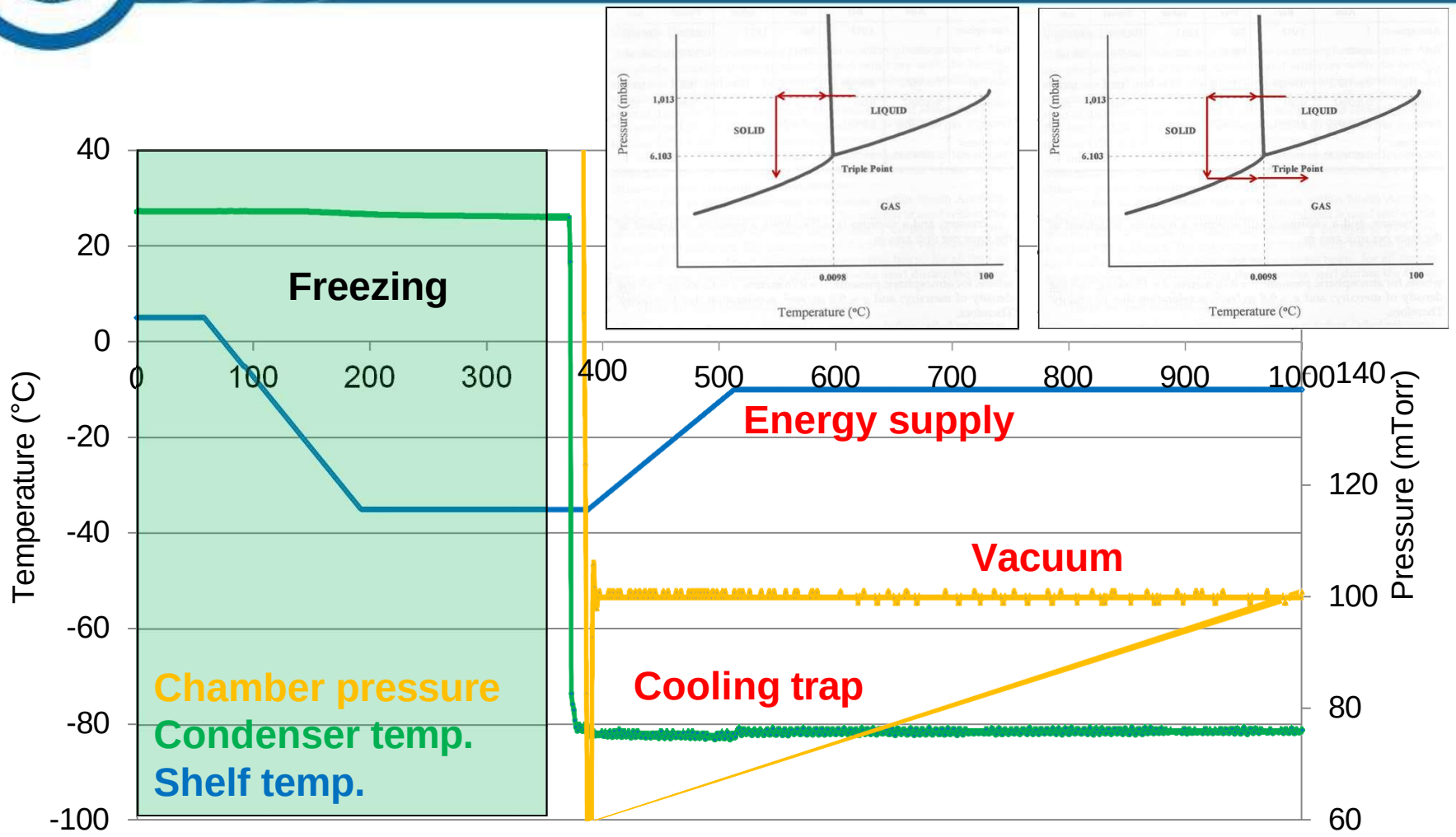
Freezing - Nucleation



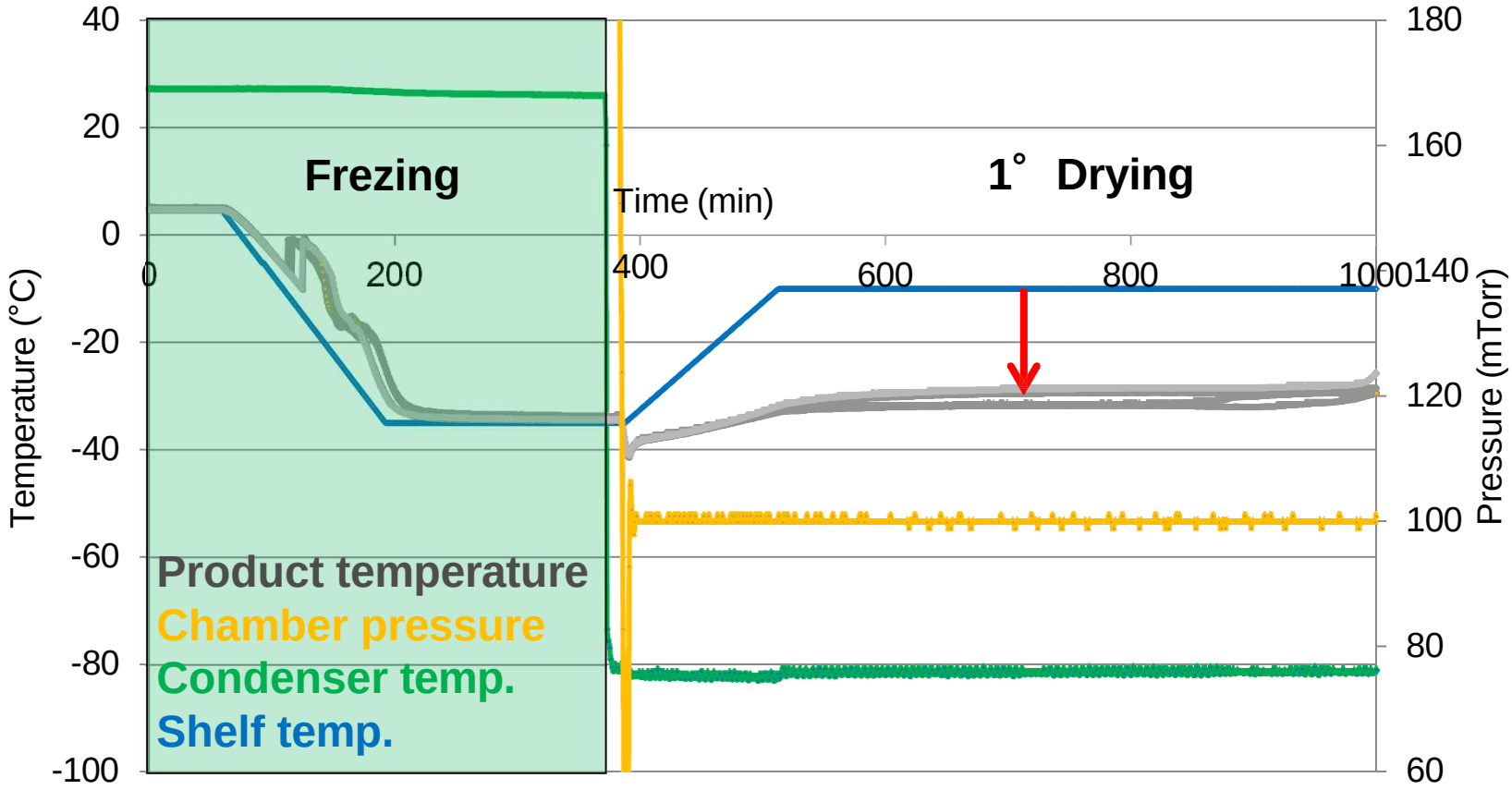
Primary Drying



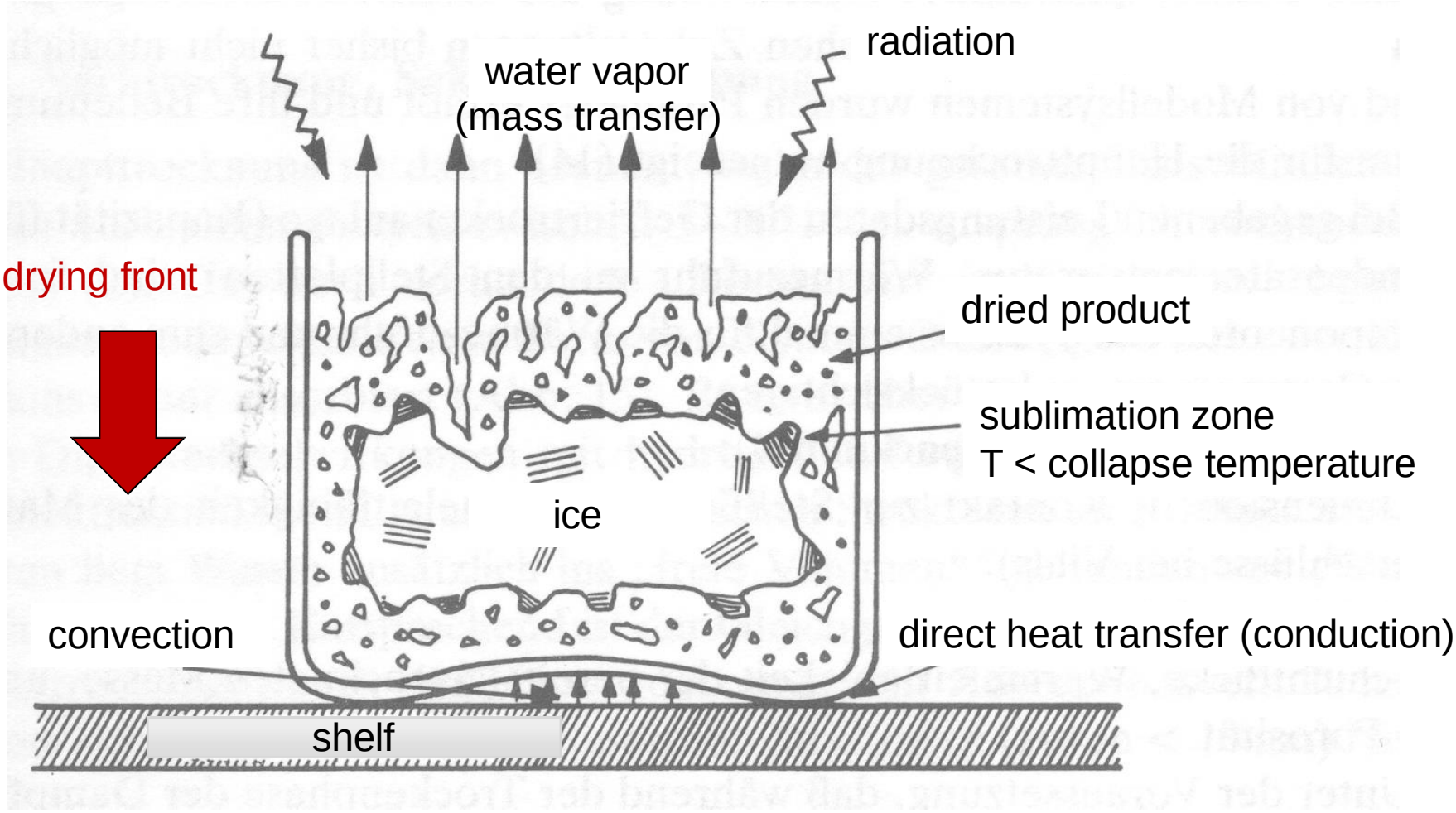
Primary Drying



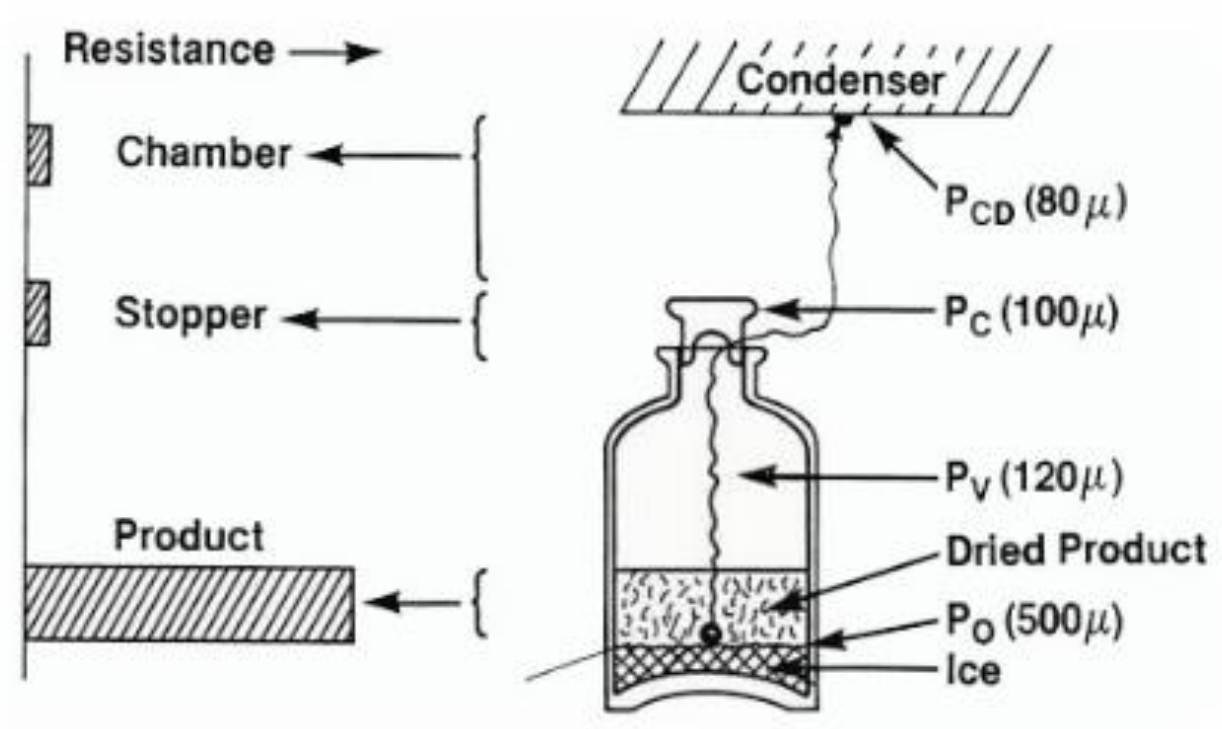
Primary Drying - Sublimation



Primary Drying - Sublimation

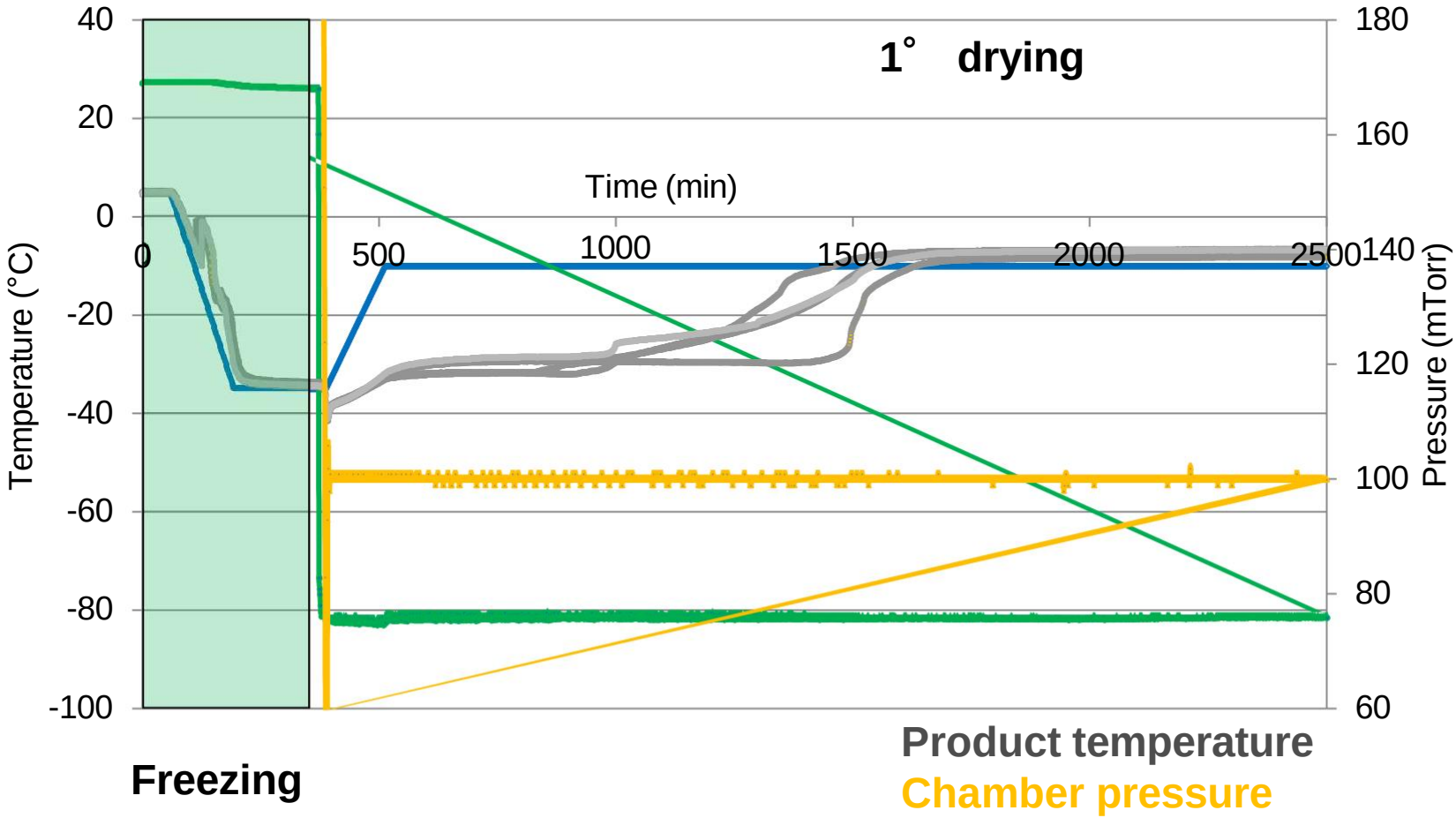


Primary Drying - Barriers to mass transfer

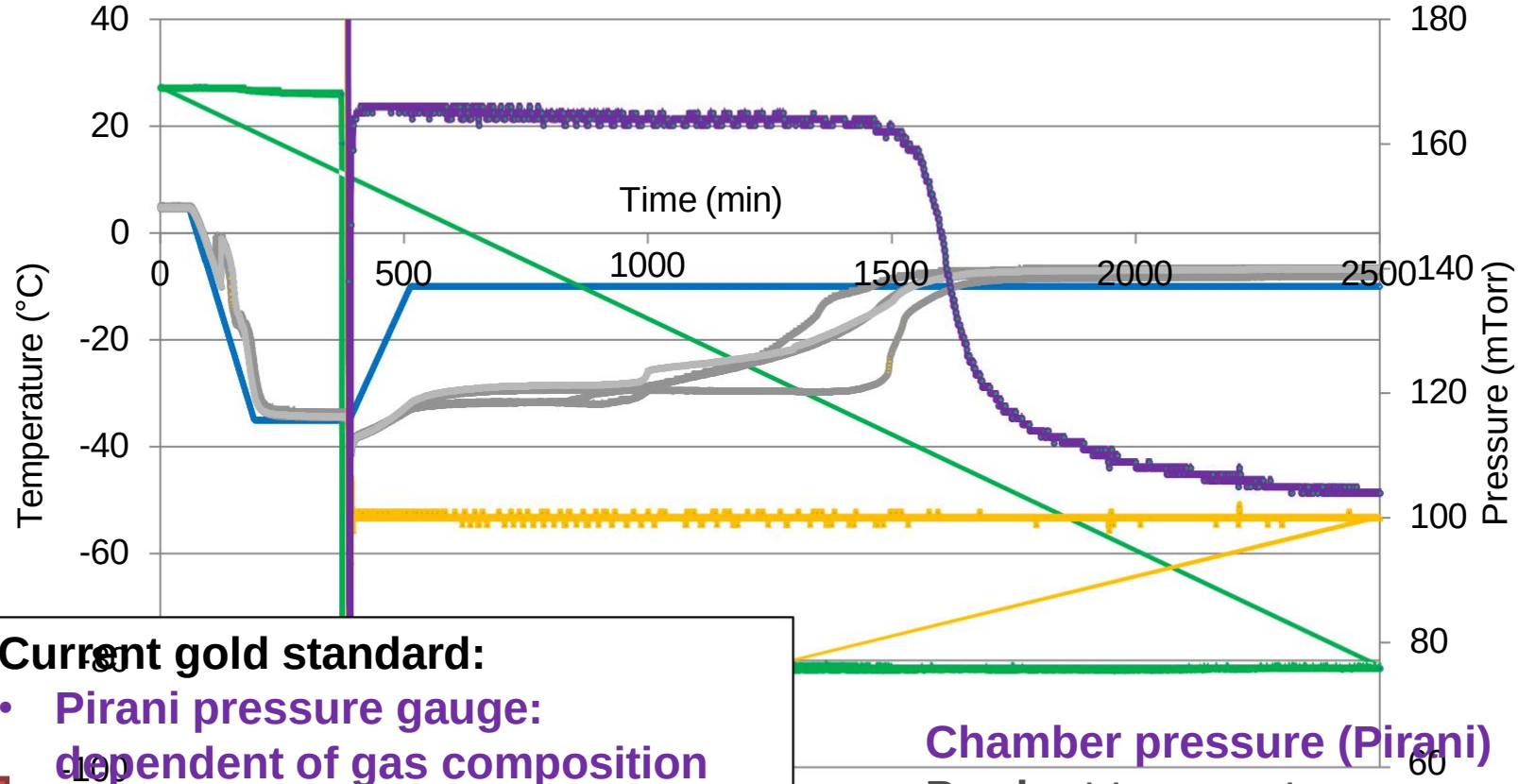


Mass transfer in primary drying. Schematic of resistances (pressure in µm Hg).
100 µg Hg = 133 µbar
P_0 – equilibrium vapor pressure of ice at sublimation interface
P_V – pressure in the vial
P_C – chamber pressure
P_{CD} – condenser pressure

End of primary drying: Product temperature



End of primary drying: Pressure gauges



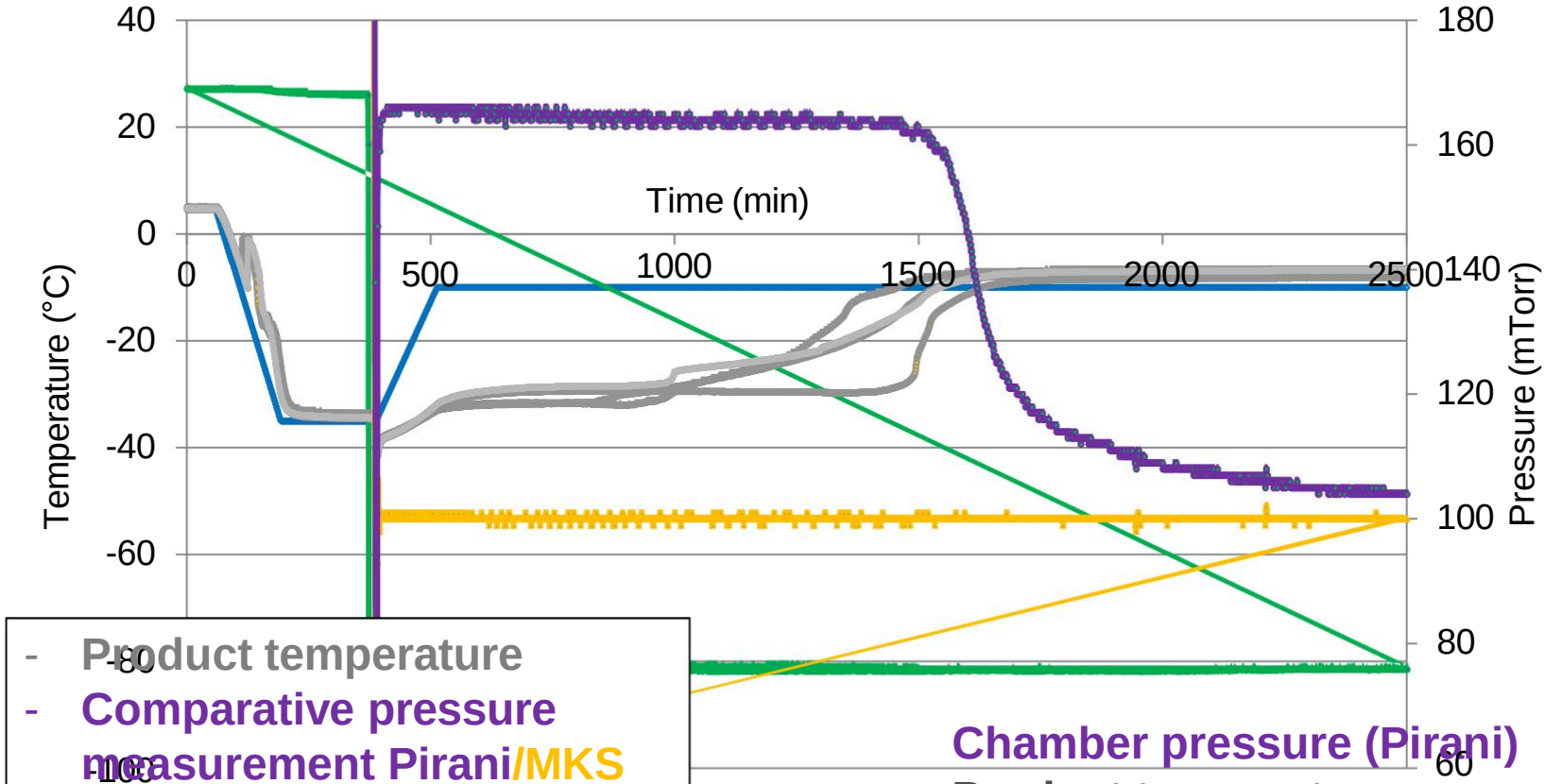
Current gold standard:

- **Pirani pressure gauge: dependent of gas composition (in the chamber)**
- **MKS pressure gauge: independent of gas composition**

Theory 4

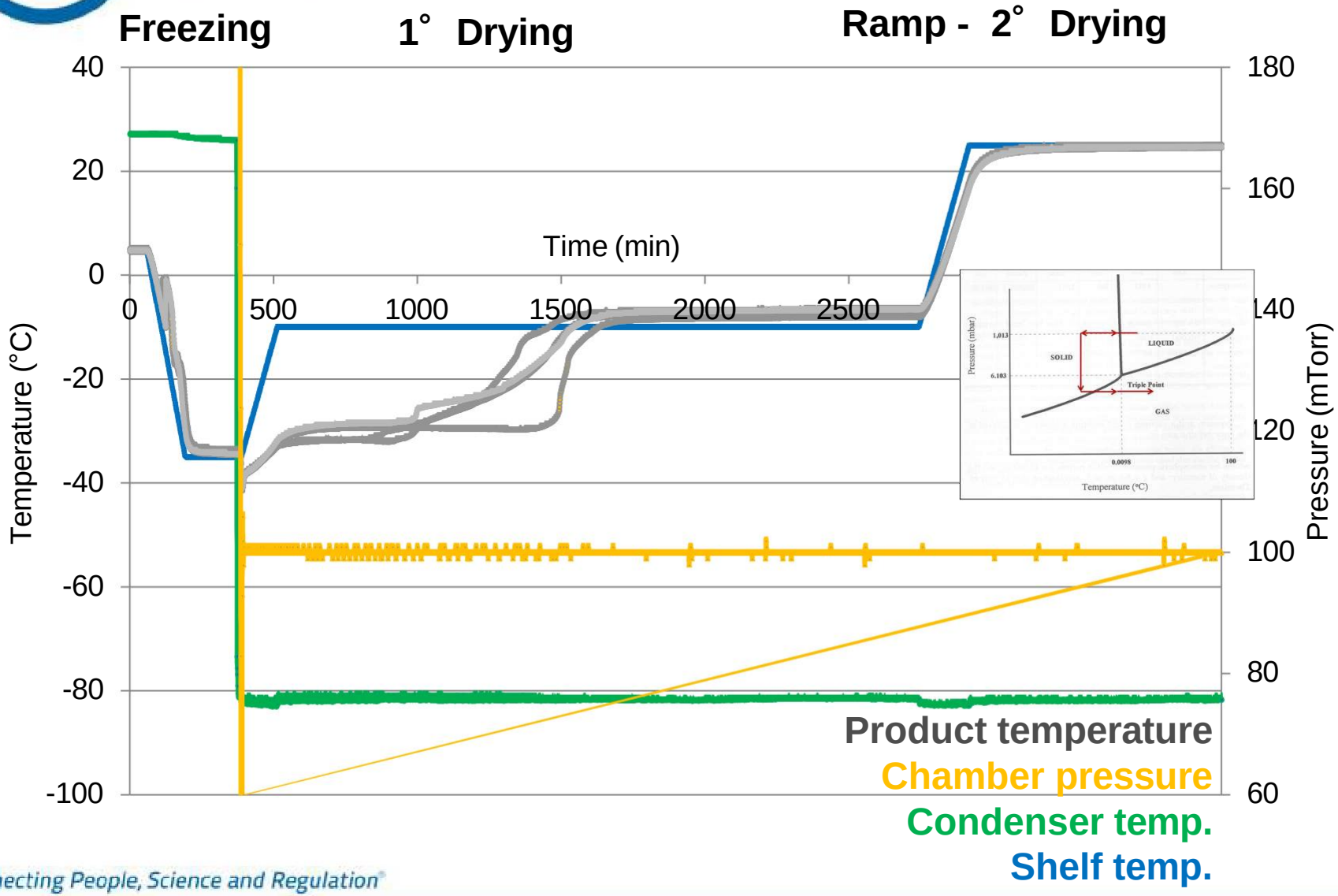
Chamber pressure (Pirani)
Product temperature
Chamber pressure (MKS)
Condenser temp.
Shelf temp.

End of primary drying - Options

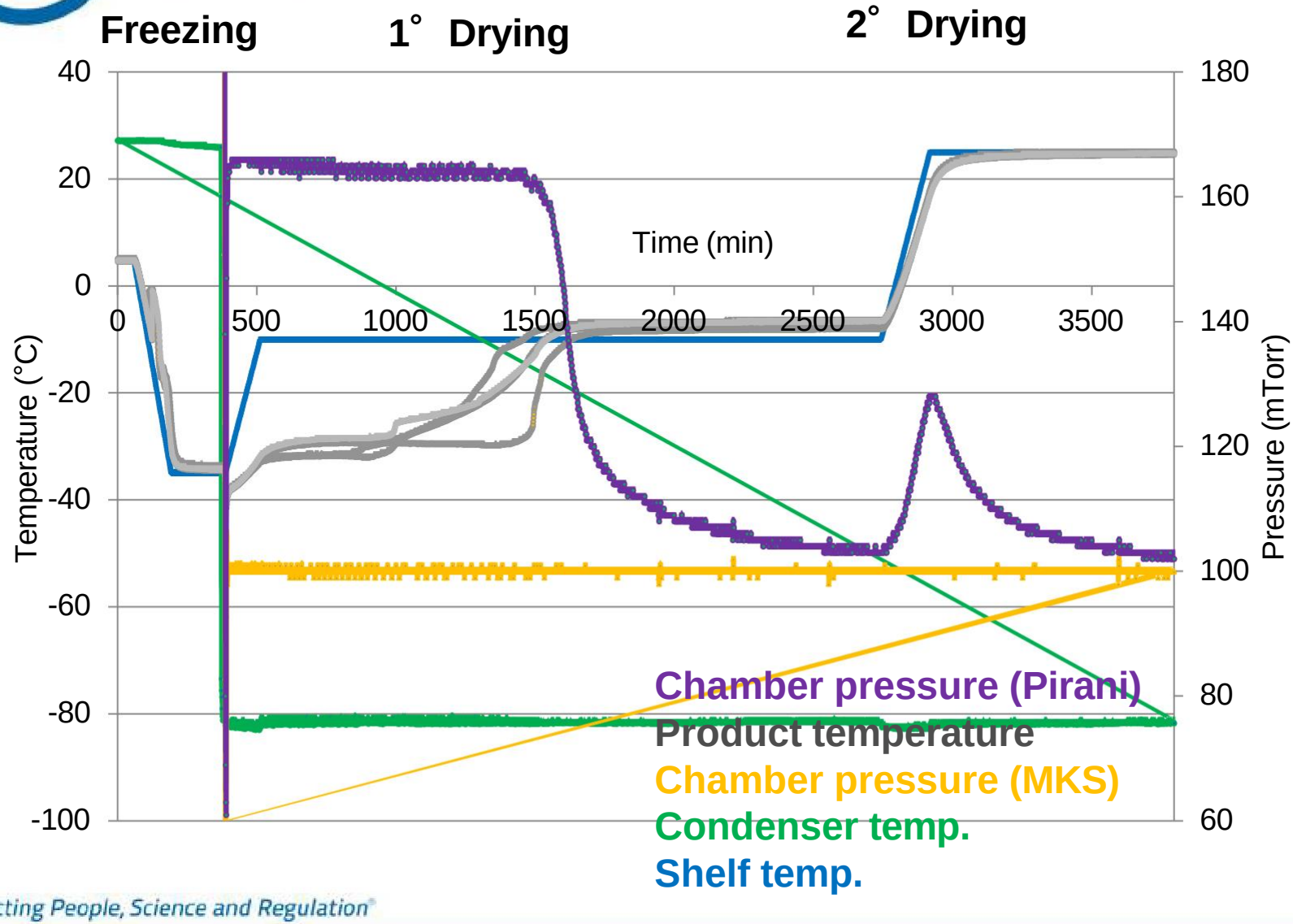


- Product temperature
- Comparative pressure measurement Pirani/MKS
- Pressure rise test

Secondary drying - Desorption

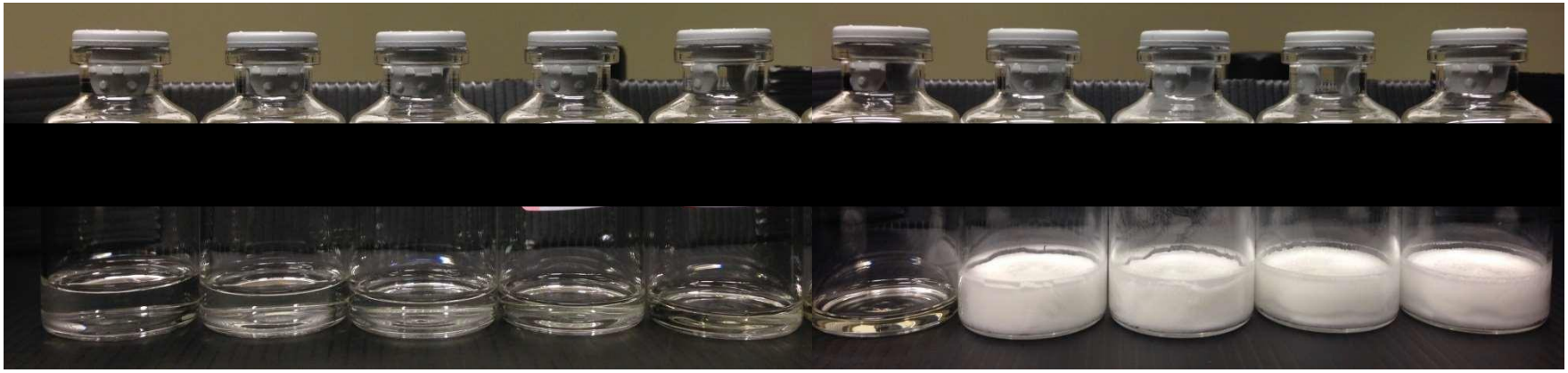


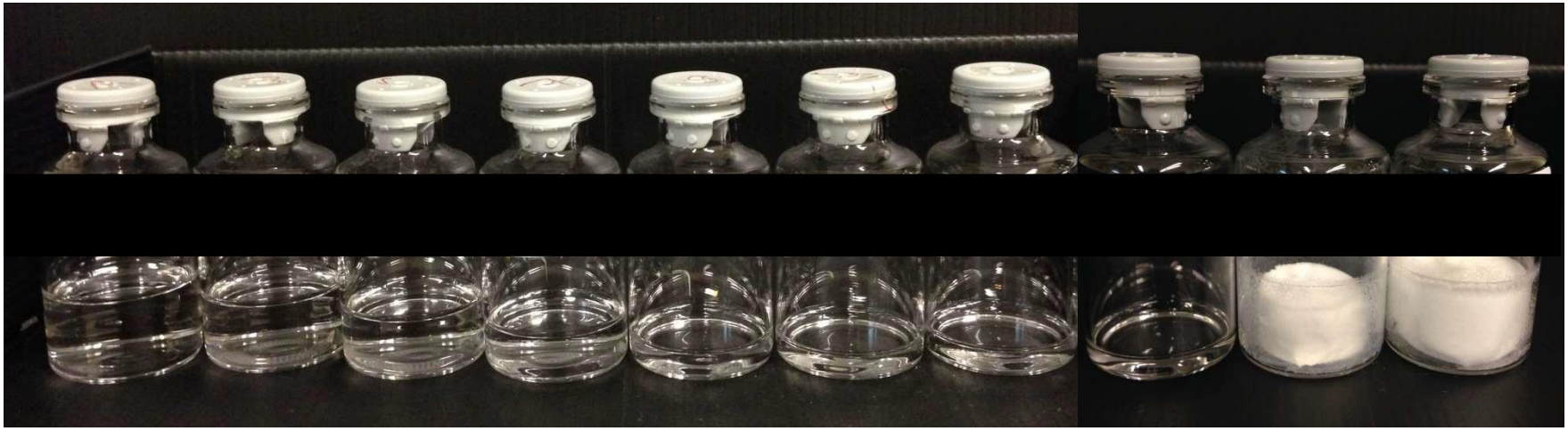
Secondary drying - Desorption



Literature recommendation: M. J. Pikal, S. Shah, M. L. Roy, and R. Putman. The secondary drying stage of freeze drying: drying kinetics as a function of temperature and chamber pressure. Int. J. Pharm. 60:203–217 (1990).

Progress of drying

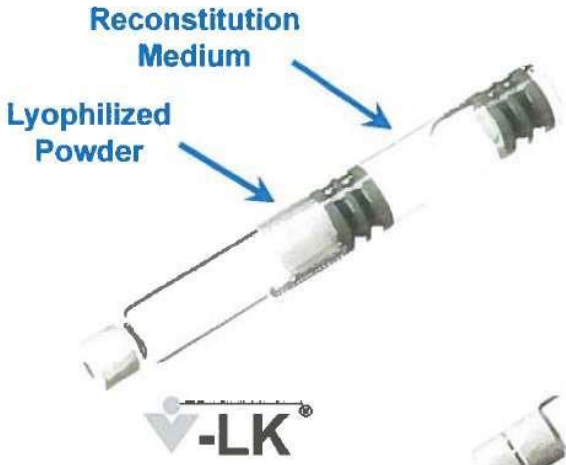




Primary packaging

Vial &
Elastomer
stoppers

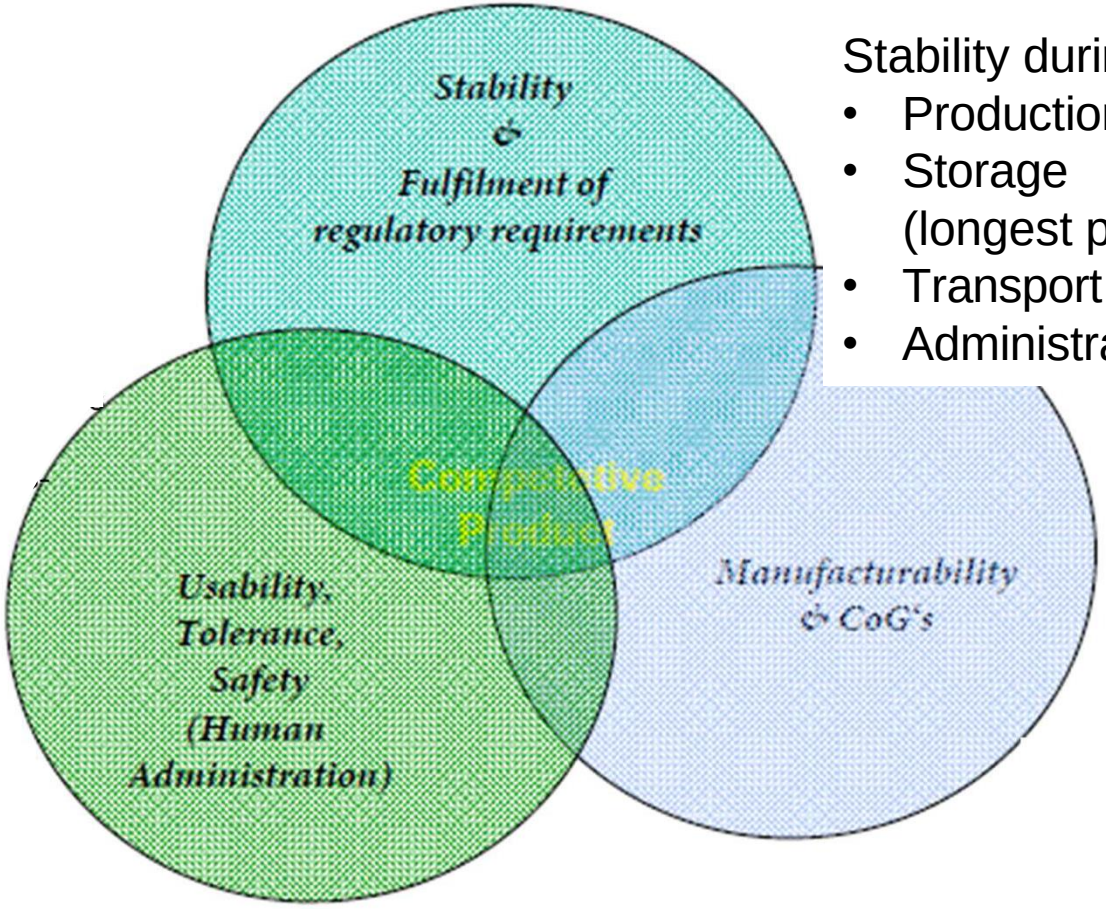
(different coatings)



Dual
chamber
Cartridge

Syringe
(Dual chamber syringe)

Requirements of a Drug Product



Stability during:

- Production
- Storage (longest possible)
- Transport and
- Administration

- Patient conveniene
- Patient adherence
- Dose delivery

Special caution with proteins: Influence on undesirable adverse events and clinical efficacy, immunogenicity and pharmacokinetic profile through product specific degradation products.

Design of a (protein) formulation

Antioxidant (Methionine)

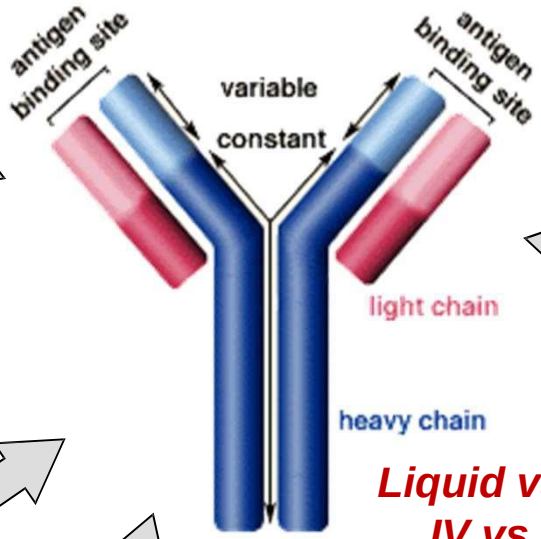
Preservatives (Multi-does-vials)

Benzylalcohol

Buffer system

(His/HisHCl, Citrate, ~~Acetat~~)

~~Phosphate~~



Lyo/cryoprotectants and bulking agents (Sucrose, Trehalose, Mannitol,…)

Stabilizers during freezing/ thawing (Sucrose, Trehalose)

- tonicity adjusting agents at the same time

Liquid vs. Lyo
IV vs. SC

Surfactants (Polysorbate 20, 80, Poloxamer)

Viscosity reduction e.g. Arginine-HCl

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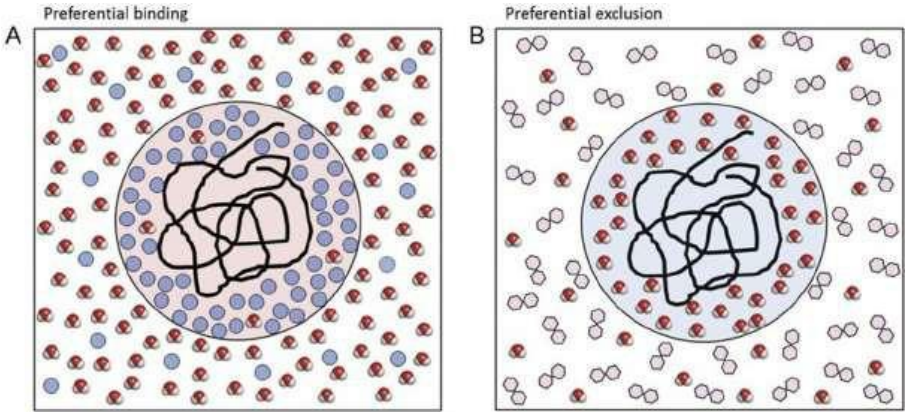
Literature recommendation: Marketed products in EU: Gervasi V, et al. Eur J Pharm Biopharm. 2018;131 (2017):8–24. & Stability of protein pharmaceuticals: Manning MC et al. Pharm Res. 2010;27(4):544–75.

Lyo/cryo-protective excipients

Cryoprotectant

Stabilizes during the freezing process

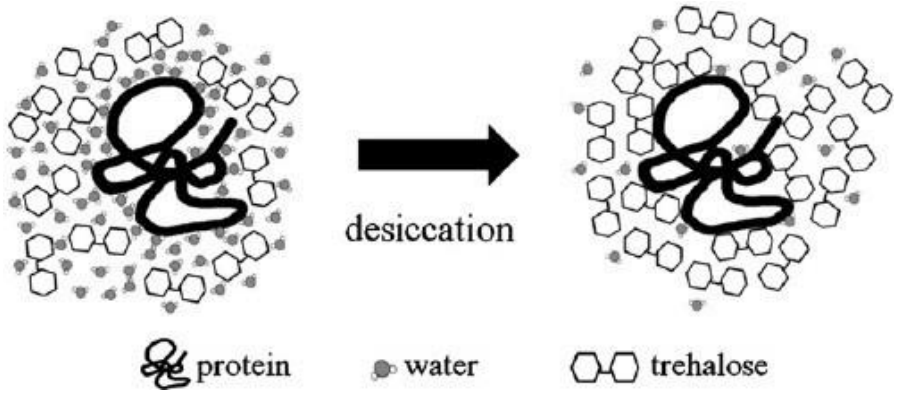
- Excipients are preferentially excluded from the surface of the protein. This is an thermo-dynamically unfavored state. As the unfolded state of the protein would enhance this state, the protein is stabilized.
- (Timasheff 1993).



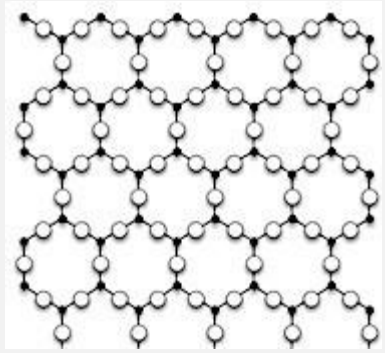
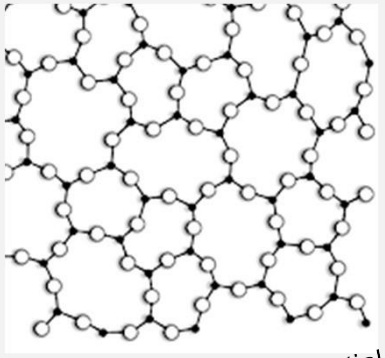
Lyoprotectant

Stabilizes during the drying process

- Water stablizes a protein in liquid solution by hydrogen bonding. The excipient replaces the hydrogen bonds of water during drying and thus stabilizes the protein.



Lyo/cryoprotective excipients

Crystalline excipients	Amorphous excipients
Ordered crystal structure 	Glassy state 
Eutectic temperature (defined melting point)	Glas transition temperature Characterization by differential scanning calorimetry

• Bulking agent
• High eutectic temperature :
  • Elegant cake appearance
  • Fast drying
	• Stabilzation of e.g. proteins
	• Acceptable bulking agent at the same time

• In many cases no stabilization (e.g. for most proteins) • Different morphologies dependent on excipient (Mannitol→ Annealing) • Glass breakage (Mannitol at high fill)	• Low M_w excipients: Low glass transition temperatures → Cake structure? • High M_w excipients: Higher glass transition temperatures → poorer stabilization?
Glycine, Mannitol, NaCl, …	Sucrose, Trehalose, HPβCD, PVP, Dextran, …

Examples

Kadcyla 100 / 160mg

20 mg/mL ado-trastuzumab emtansine
10 mM sodium succinate pH 5.0
60 mM D-Sucrose
0.02% Polysorbate

Herceptin 150 / 400 mg

25 mg/mL Trastuzumab
5 mM L-Histidine/-HCl, pH 6.0
60 mM D-Trehalose
0.01 % Polysorbate 20

Analytical characterization

Product attributes for designing lyophilization cycles

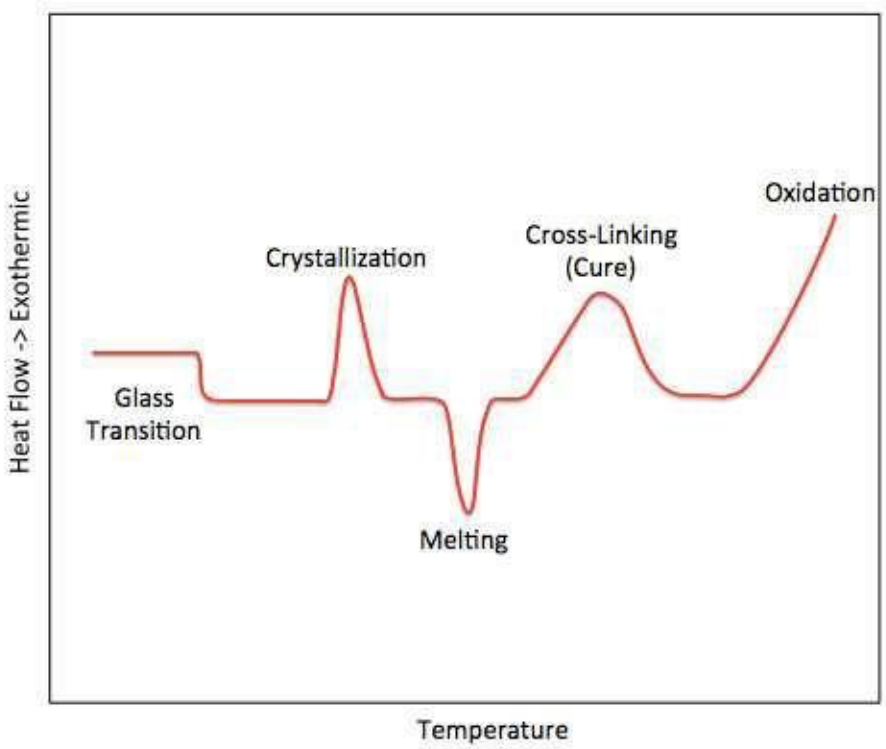
- Differential scanning calorimetry: $T_{g'}$, T_g, T_{eut}
- Freeze drying microscopy: $T_{collapse}$

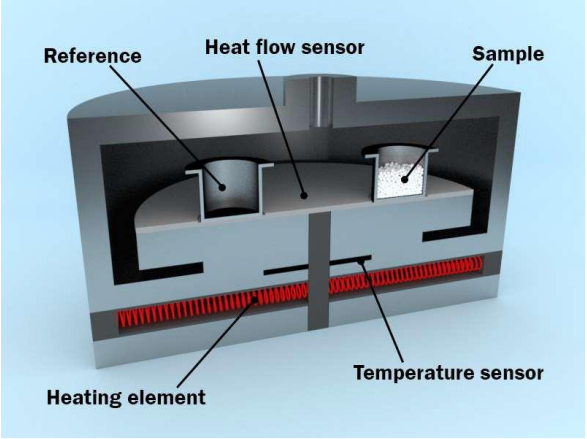
Solid state characterization after lyophilization

- Residual moisture (Karl Fischer, NIR, FMS)
- Reconstitution time
- Thermodynamic / Solid state (X-ray powder diffraction)
- Specific surface area (BET)
- Cake appearance at different levels

Other quality attributes of active compound

Differential Scanning Calorimetry (e.g. $T_{g'}$)

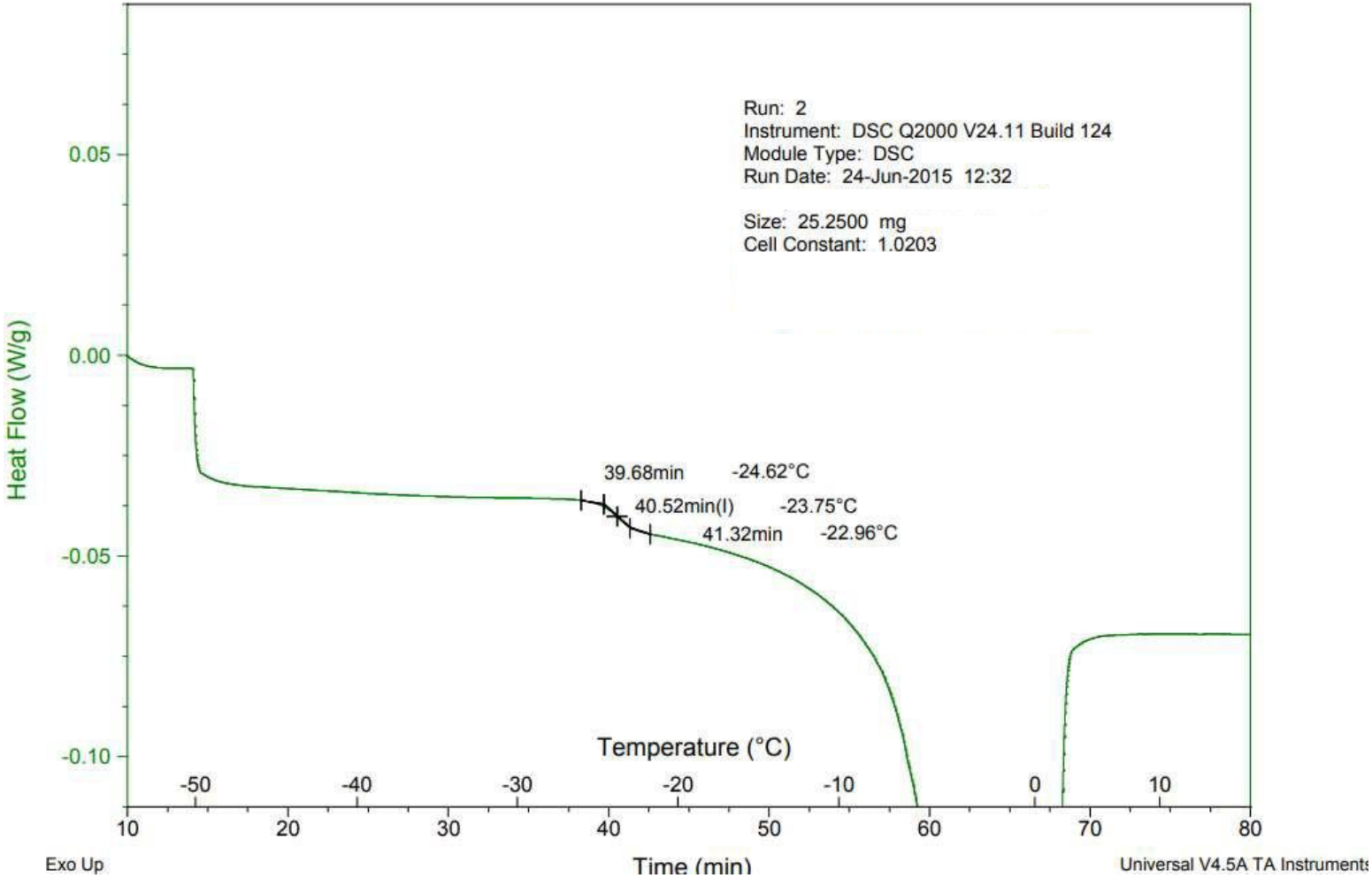




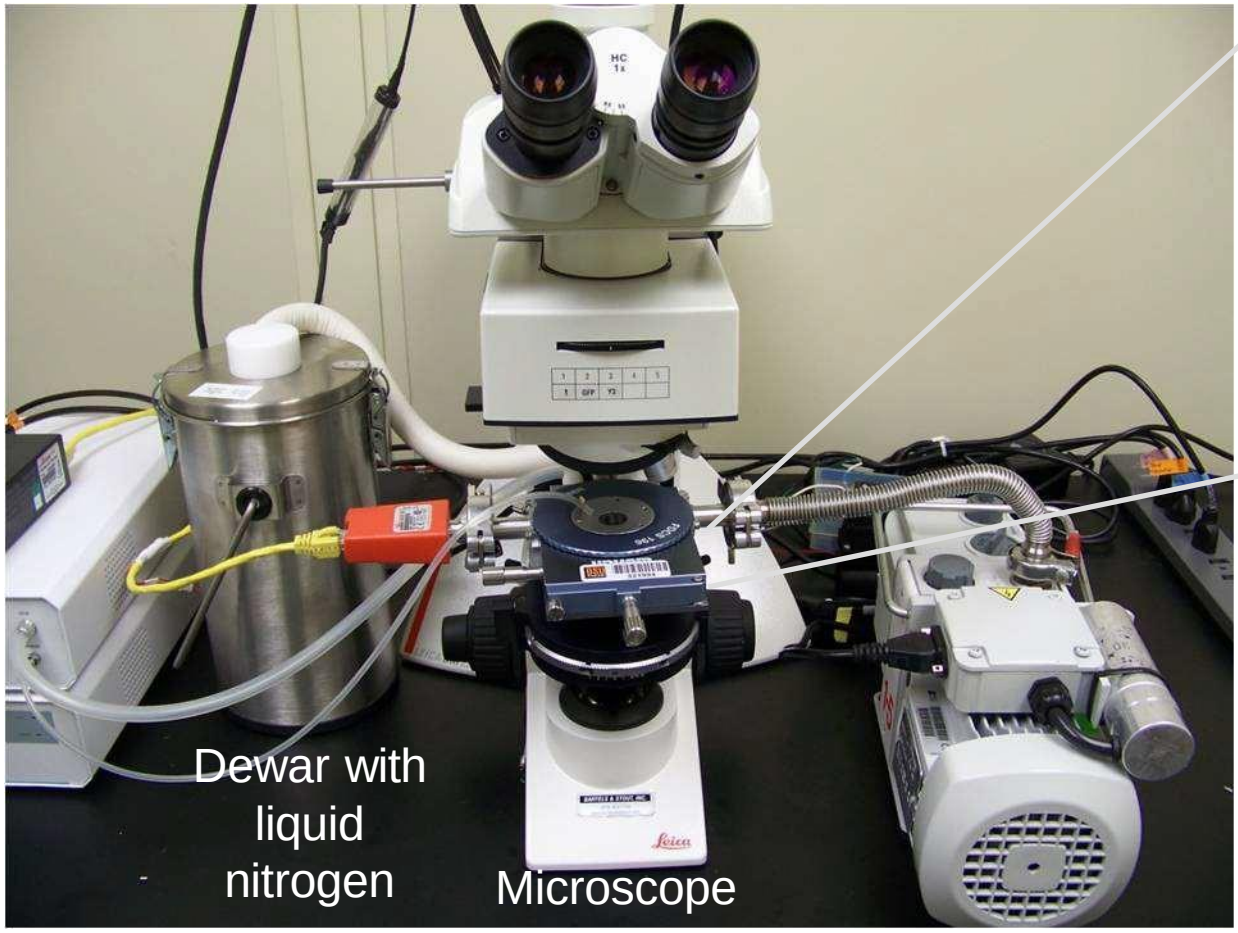
- Thermal analysis to detect physical transformation such as phase transitions (e.g. glass transition temperature $T_{g'}/T_g$, crystallization/melting point T_{eut} …)
- Measurement of the difference in the amount of heat required to increase the temperature of a sample compared to a reference with well-defined heat capacity as a function of temperature
- Both the sample and reference are maintained at nearly the same temperature throughout the experiment

Differential Scanning Calorimetry (e.g. T_g')

Tg' = Glass transition temperature of the maximally freeze-concentrated solution



Freeze drying microscopy ($T_{collapse}$)

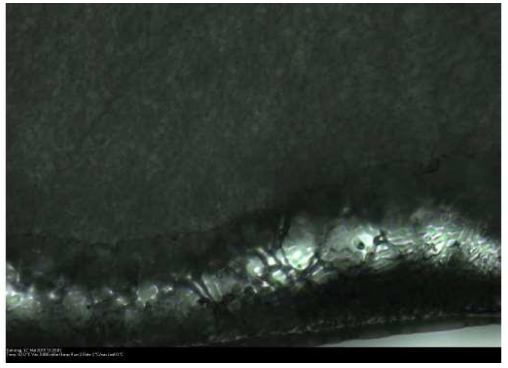


Cryostage

Freeze drying microscopy ($T_{collapse}$)

(Intact) frozen sample

Onset of collapse



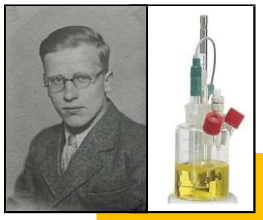
Complete collapse

→ $T_{g'} < T_{collapse}$!!

Residual moisture – Water content

Gravimetric analysis

- Loss of mass in drying cabinet (TGA) or IR
- Targets any volatile components
- Destructive
- Does not account for 'hidden' water



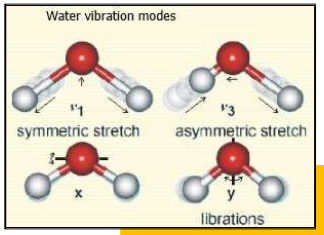
Karl-Fischer titration

- Quantitative water determination by titration

$$CH_3OH + SO_2 + RN \rightleftharpoons (RNH)SO_3CH_3$$

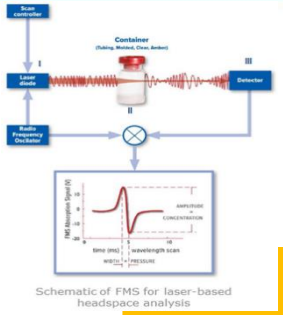
$$H_2O + I_2 + (RNH)SO_3CH_3 + 2\,RN \rightleftharpoons (RNH)SO_4CH_3 + 2\,(RNH)I$$

- Destructive
- Volumetric versus coulometric
- Extraction versus direct measurement



NIR spectroscopy

- Fingerprinting of molecule vibrations by near infrared
- Non-destructive
- High throughput (can be automated)
- Model generation and multivariate calibration techniques needed (e.g., principal components and partial least square analysis)



Headspace analysis w/ FMS

- Measures absorption of laser light (1400 nm) and converts it to water vapor pressure
- Non-destructive
- High throughput (can be automated)
- Vial format-specific calibration needed
- Water vapor pressure can be translated into cake moisture via Karl Fischer correlation (equilibration time!)

Karl-Fischer Titration

- Two media are needed: Titrating agent and working medium consisting of the three components sulfur dioxide, alcohol, and organic base or/and water free vehicle.
- End-point detection occurs either by color change or potentiometrically via an indicator electrode (free I_2/I- redox couple).

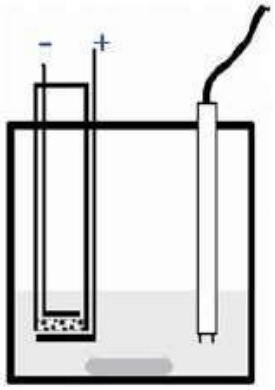
Volumetric Karl Fischer Titration

Iodine is added by a burette during titration.
Suitable for samples where water is present as a major component: **100 ppm - 100%**

$$CH_3OH + SO_2 + RN \rightleftharpoons (RNH)SO_3CH_3$$

$$H_2O + I_2 + (RNH)SO_3CH_3 + 2\,RN \rightleftharpoons (RNH)SO_4CH_3 + 2\,(RNH)I$$

Redox reaction



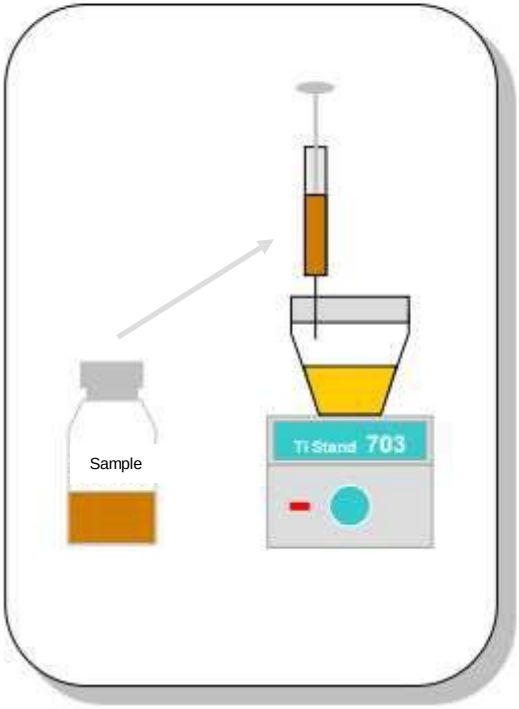
Coulometric Karl Fischer Analysis

Iodine is generated electrochemically during titration.
Suitable for samples where water is present in trace amounts: **1 ppm - 5%**

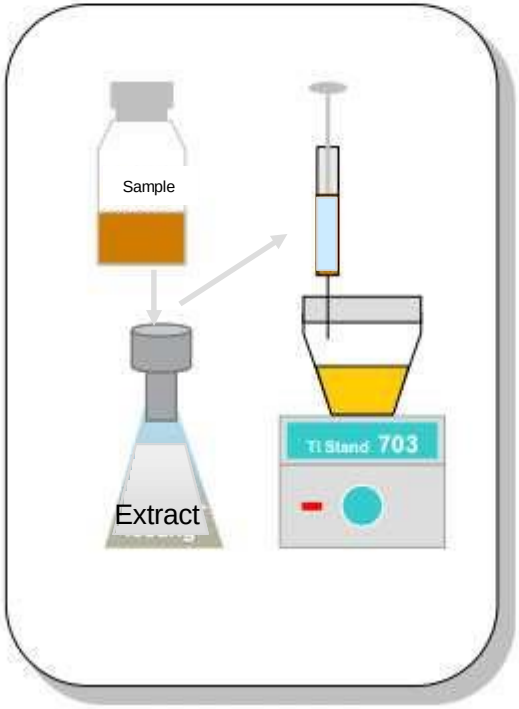
- The working medium consists of the components sulfur dioxide, alcohol, and organic base or/and water free vehicle.
- Two electrodes are needed: One for Iodine generation (anode), and one for potentiometric end-point detection via the indicator electrode (free I_2/I- redox couple).

Karl-Fischer Titration

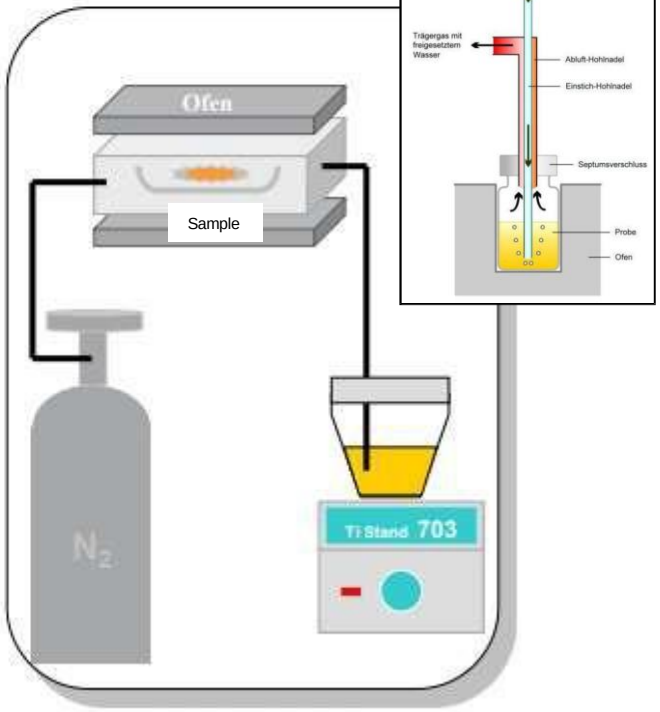
Direct Titration



Liquid Extraction

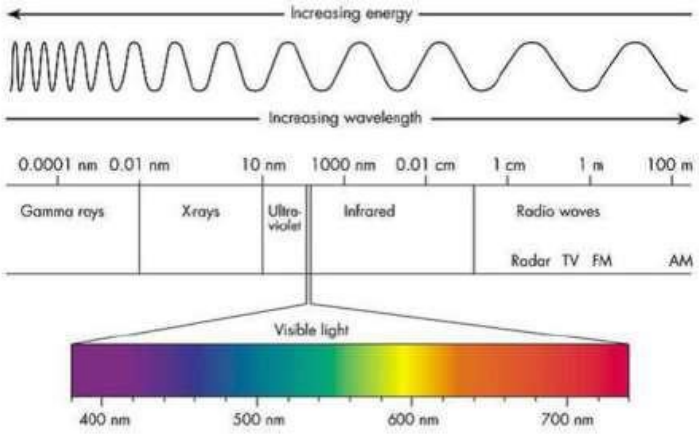


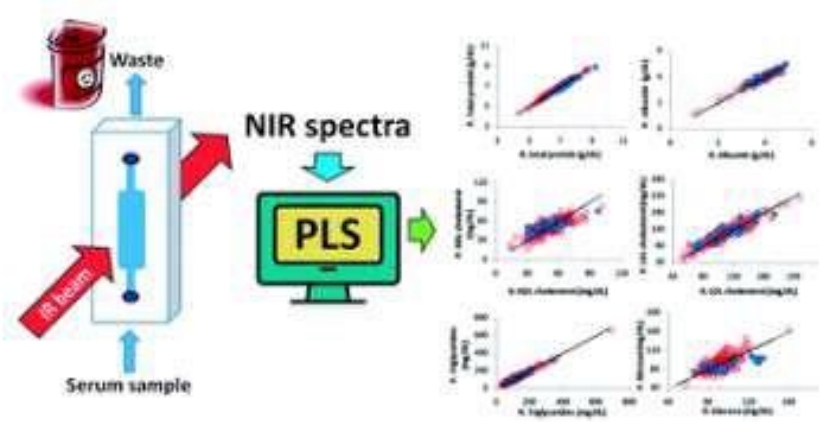
Evaporation

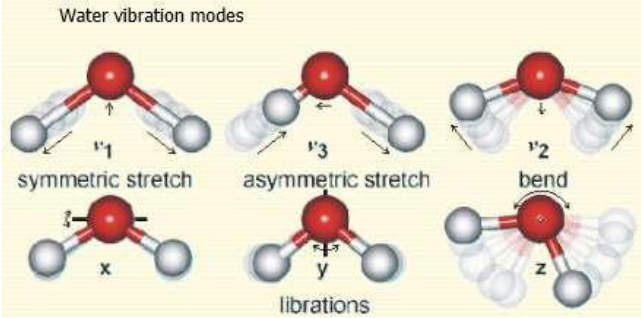


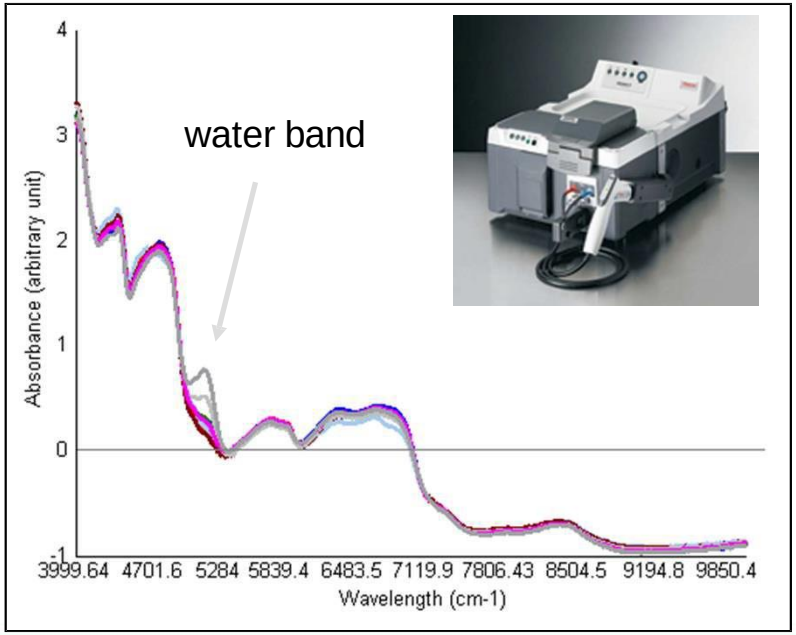
Highly dependent on the sample and its heat sensitivity.

Residual moisture - NIR









- Molecule vibrations (overtone and combinations)
- Near infrared: ~760–2500 nm or 13.000–4.000 cm−1

Analytical characterization

Product attributes for designing lyophilization cycles

- Differential scanning calorimetry: $T_{g'}$, T_g, T_{eut}
- Freeze drying microscopy: $T_{collapse}$

Solid state characterization after lyophilization

- Residual moisture (Karl Fischer, NIR)
- Reconstitution time
- Thermodynamic state (Xray powder diffraction)
- Specific surface area (BET)
- Cake appearance at different levels
  (visual inspection, 3D scanning, PDMS embedding, SEM, µCT)

Other quality attributes of active compound

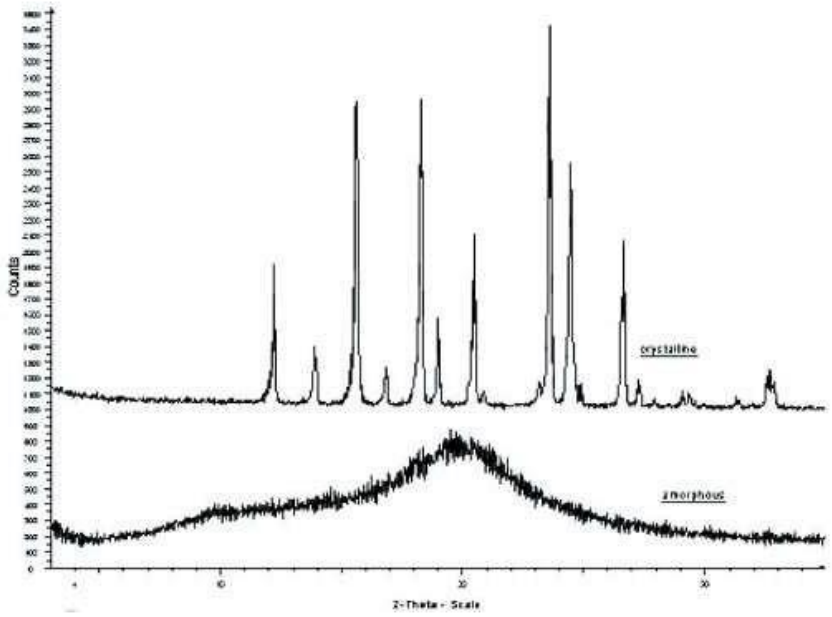
Reconstitution time

→ Water ideally flows along the side wall

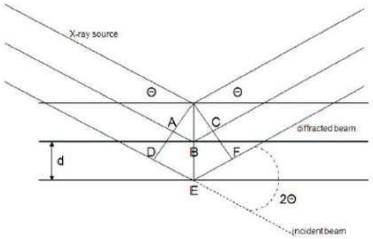
→ Avoid foaming if samples contain surfactants

→ In case of long reconstitution times, swirling systems may be considered (no shaking!)

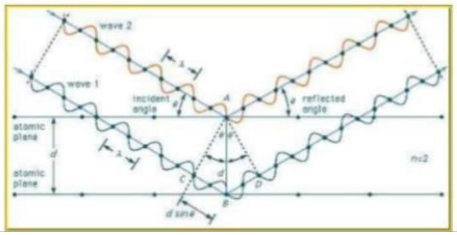
Xray powder diffraction - Morphology



The constructive and destructive interference can be measured as different intensities in the X-ray beam at given angles.



- A crystalline powder contains many small crystallites, ideally randomly oriented
- Diffraction occurs when crystallites are oriented such that specific atomic planes are in the correct relationship with the incoming x-rays

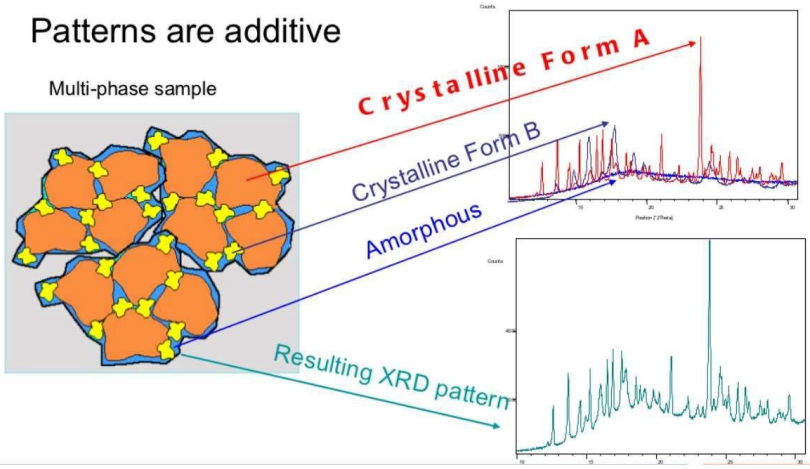


Bragg's law:
$n\lambda=2d\sin\theta$

Constructive interference is detected when the path-length difference is equal to an integer number of wavelengths

Mixture analysis

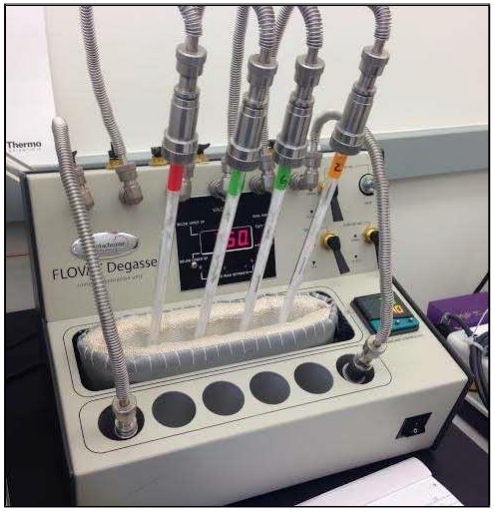
Patterns are additive

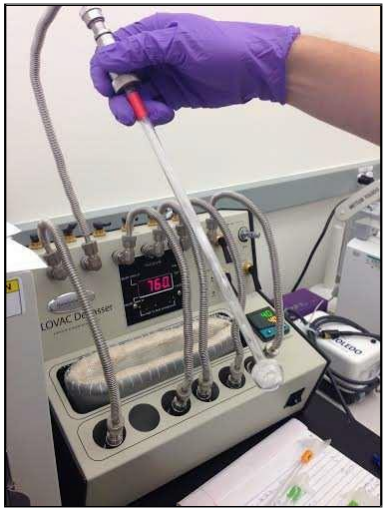


Specific surface area (BET)

S.Brunauer, P.Emmett, E.Teller Adsorption of Gases in Multimolecular Layers, J. Am. Chem. Soc., 1938, 60 (2), pp 309–319

- Physical adsorption of a gas on the surface of the solid.
- Physical adsorption results from relatively weak forces (van der Waals forces) between the adsorbed gas molecules and the adsorbent surface area of the test powder. Thus, the determination is usually carried out at the temperature of liquid N2.
- Traditionally nitrogen or helium is used as adsorbate gas.
- Based on the BET theory, the amount of adsorbed gas corresponds to a monomolecular layer on the surface.
- The amount of adsorbed gas is correlated to the total surface area of the particles including pores.





Sample preparation: degasing under vacuum and elevated temperature followed by measurement in liquid N2.

Visual inspection

Patel et al: Lyophilized Drug Product Cake Appearance: What Is Acceptable?
Patel S, Nail S, Pikal M, Geidobler R, Winter G, Hawe A, Davagnino J, Rambhatla Gupta S.
J Pharm Sci. 2017 Jul;106(7):1706-1721. doi: 10.1016/j.xphs.2017.03.014.

Cosmetic defects versus impact on product quality?

Intact cake

light collapse/melt-back

severe collapse/melt-back

complete collapse/melt-back

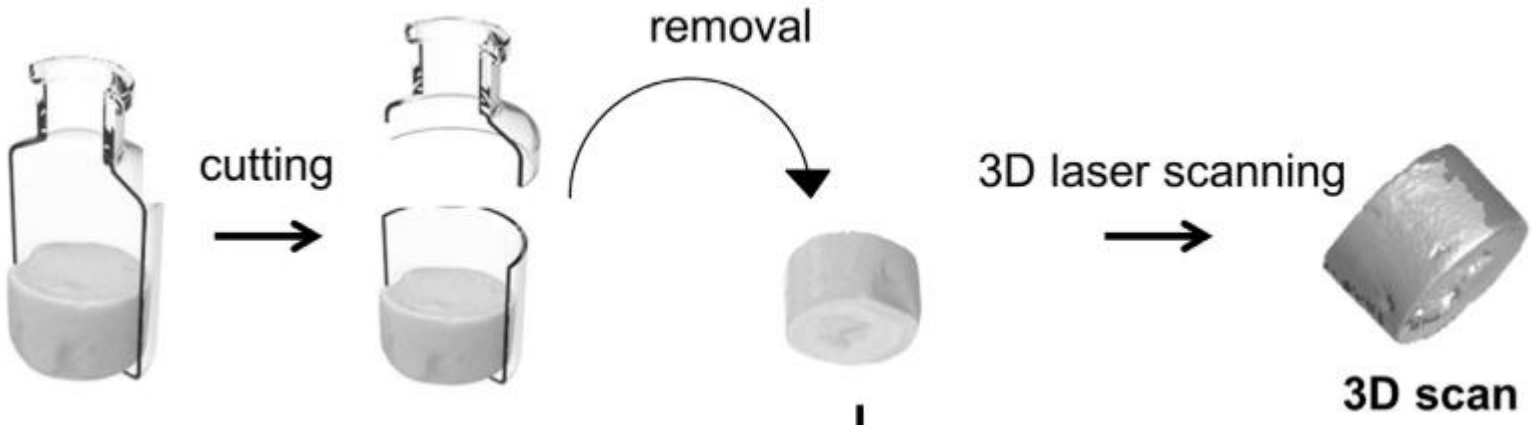
crack

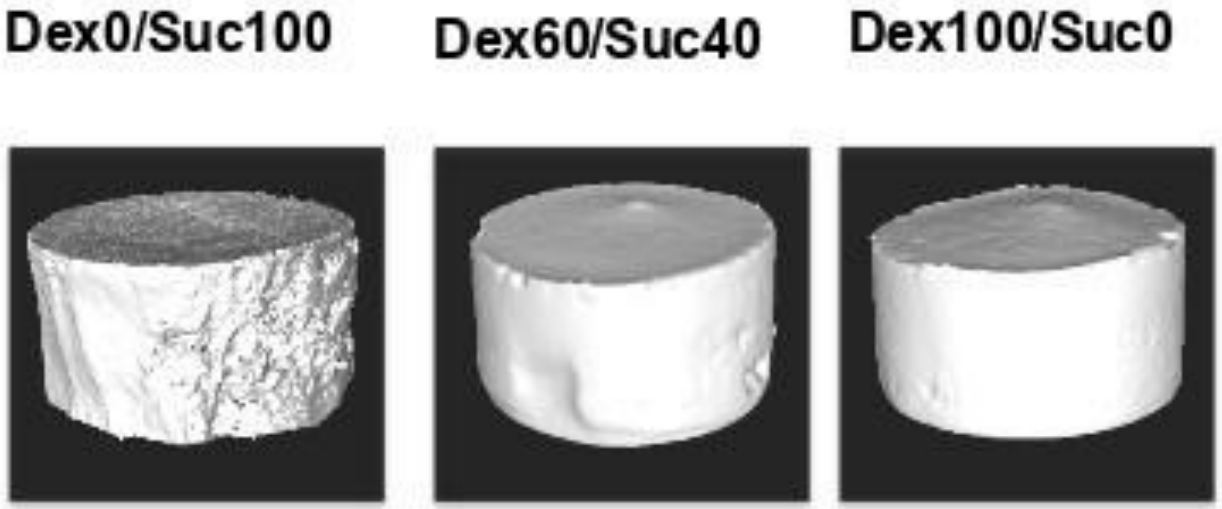
dents

splashing

fogging

3D scanning





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Literature recommendation: Haeuser C, Goldbach P, Huwyler J, Friess W, Allmendinger A.
Imaging Techniques to Characterize Cake Appearance of Freeze-Dried Products. J Pharm Sci. 2018;107(11):2810–22.

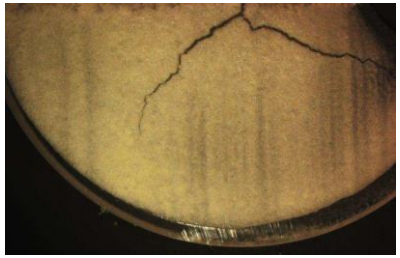
PDMS embedding

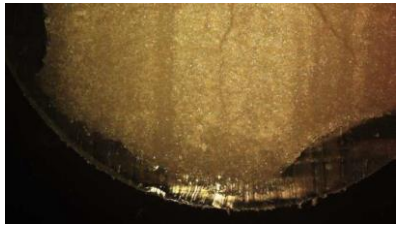
PDA Journal
of Pharmaceutical Science and Technology

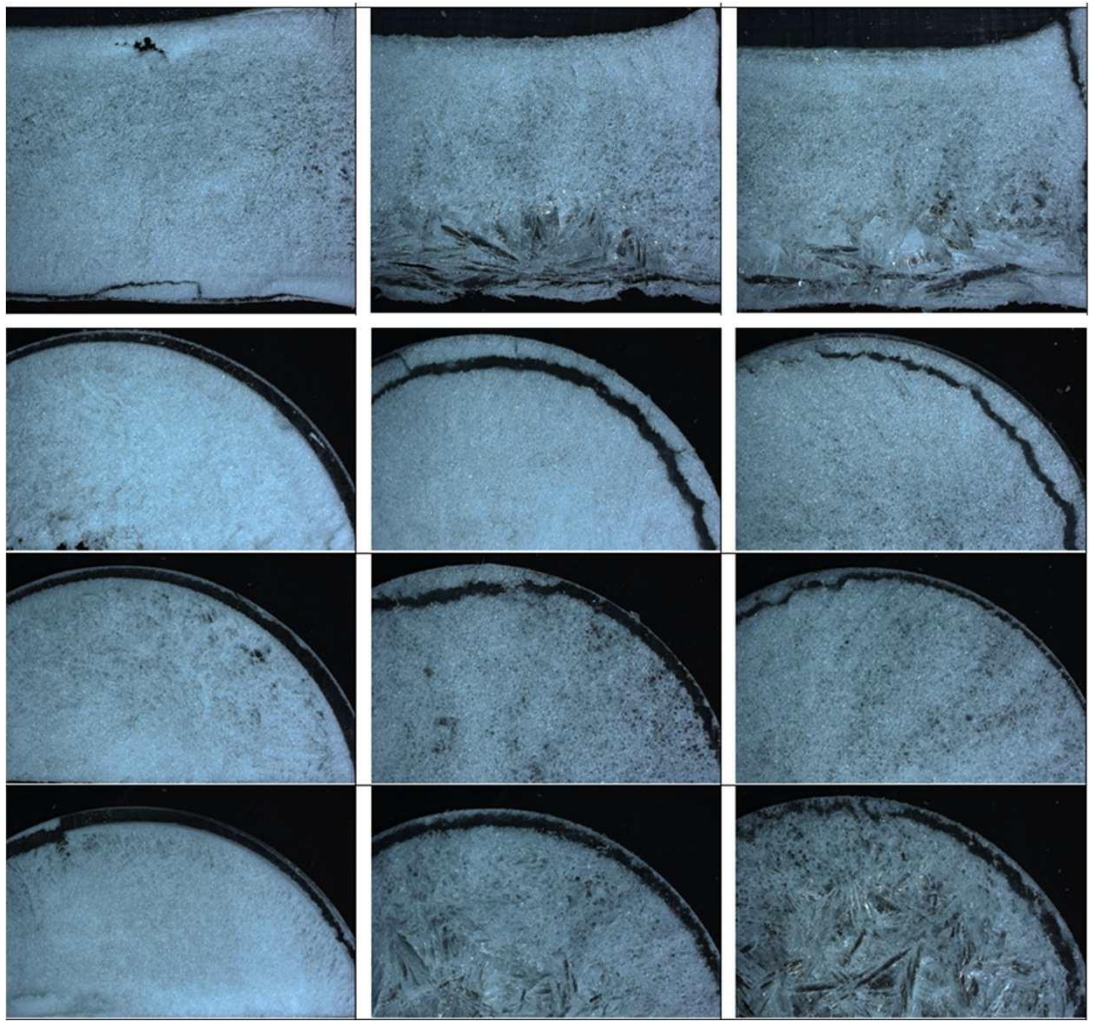
An Improved Method for Visualizing the Morphology of Lyophilized Product Cakes

Philippe Lam and Thomas W. Patapoff

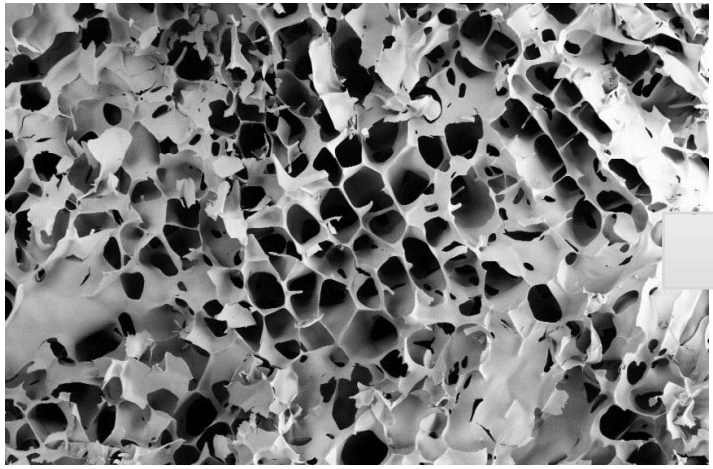
PDA J Pharm Sci and Tech **2011**, *65* 425-430

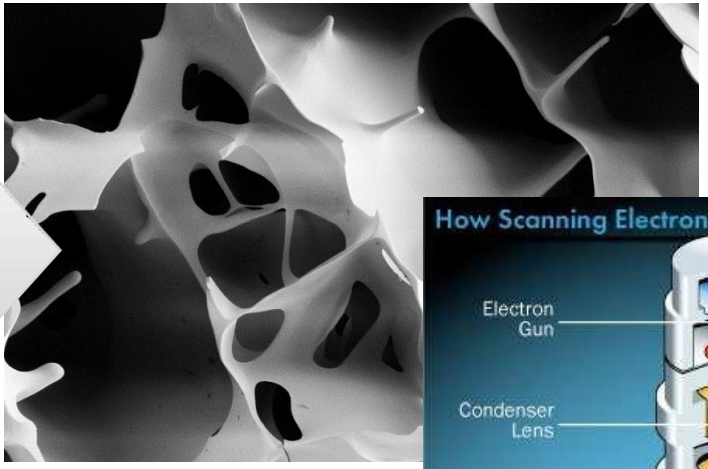


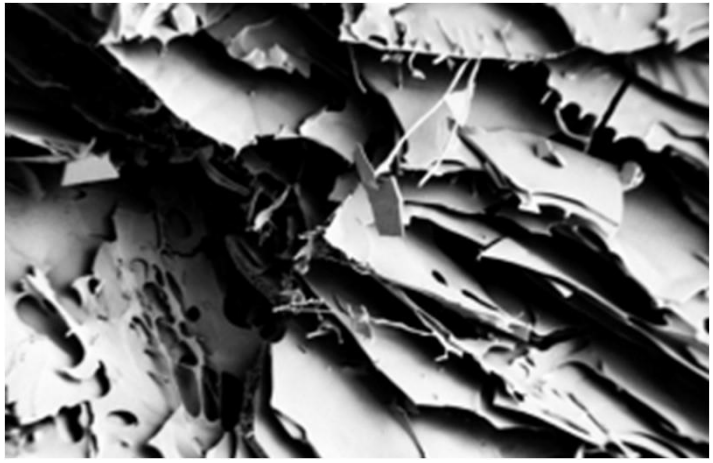


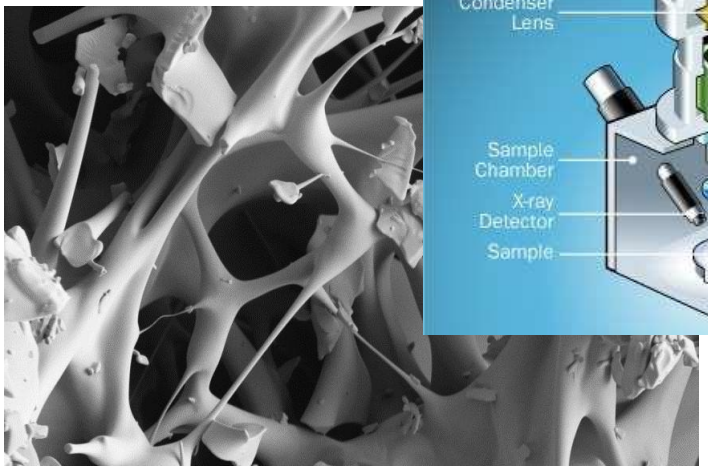


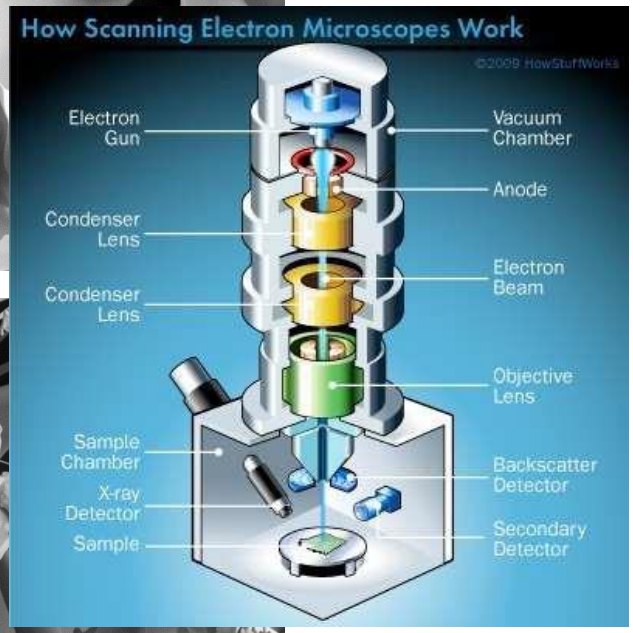
Scanning electron microscopy (SEM)



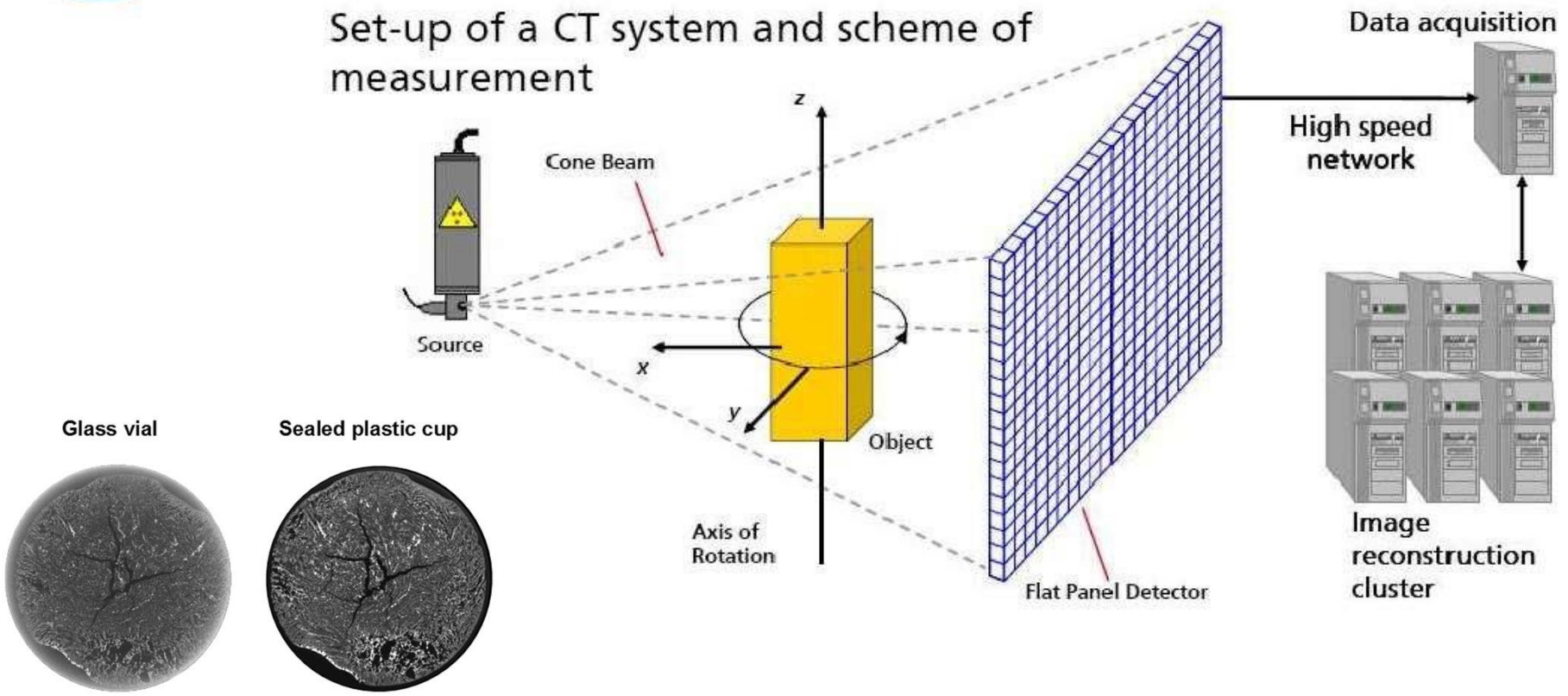






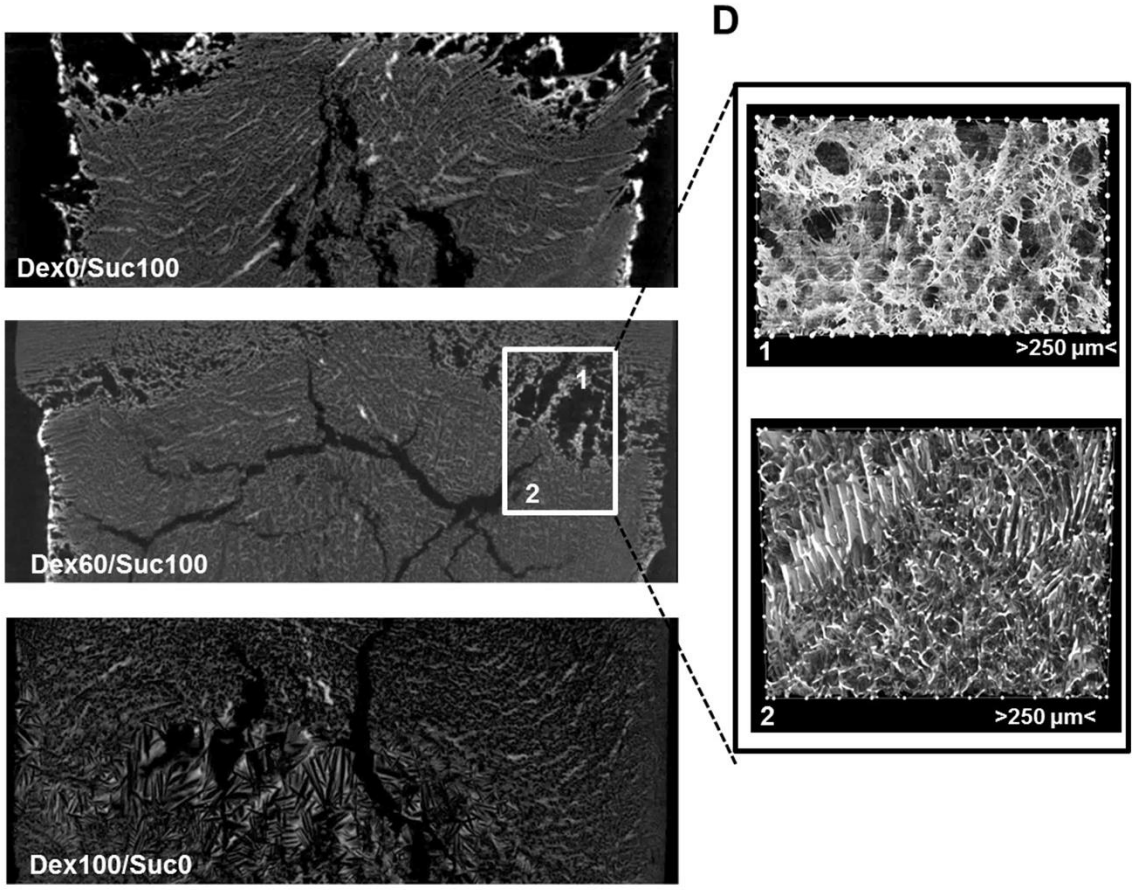


Micro-computated tomography (µCT)



- A micro-focus x-ray source illuminates the object and a planar x-ray detector collects magnified projection images.
- Based on hundreds of angular views acquired while the object rotates, a computer synthesizes a stack of virtual cross section slices through the object.
- You can then scroll through the cross sections, interpolating sections along different planes, to inspect the internal structure.
- Selecting simple or complex volumes of interest, you can measure 3D morphometric parameters and create realistic visual models.

Micro-computated tomography (µCT)



Microcollapsed structure

Intact sponge-like structure

Pros and cons and applicability of different imaging techniques summarized in Häuser et al: Imaging techniques to characterize cake appearance of freeze-dried Products. J Pharm Sci. 2018.