Theory 3

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PDA EU00144
Freeze-Drying in Practice
9 – 13 September 2024

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Osterode am Harz, Germany

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PDA Parenteral Drug Association

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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 (LOD, Karl Fischer, NIR, FMS)
  - Thermodynamic / Solid state (X-ray powder diffraction)
  - Specific surface area (BET)
  - Cake appearance at different levels (visual inspection, SEM, µ-CT)
  - Reconstitution

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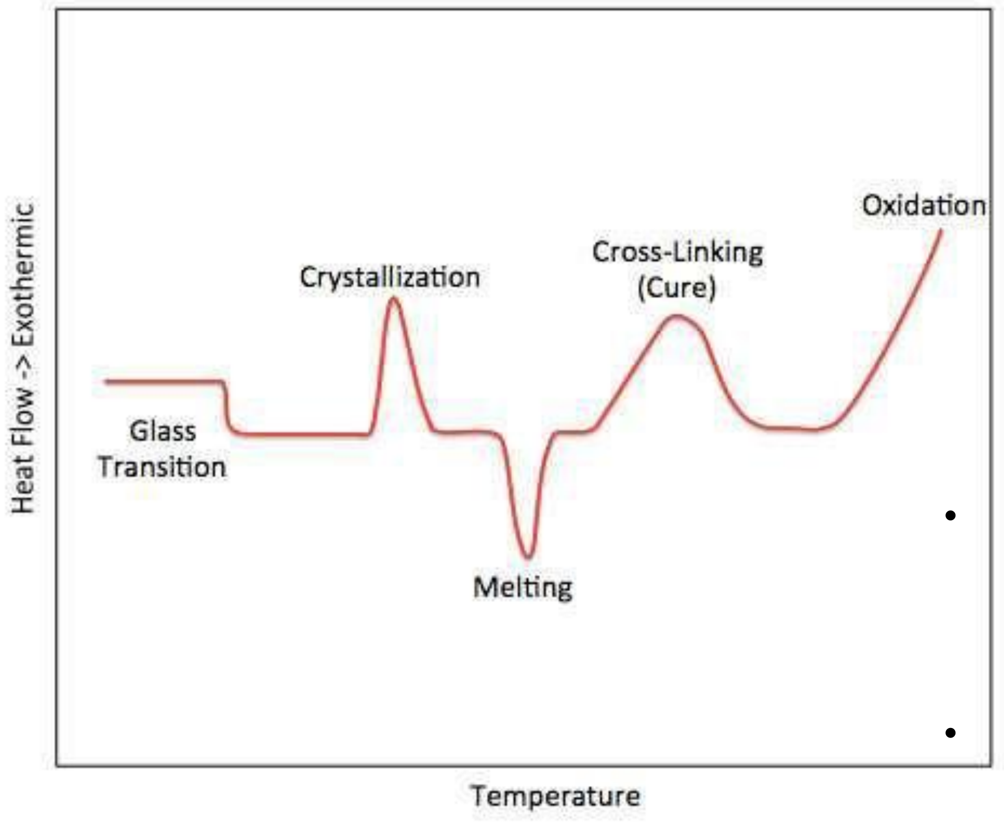
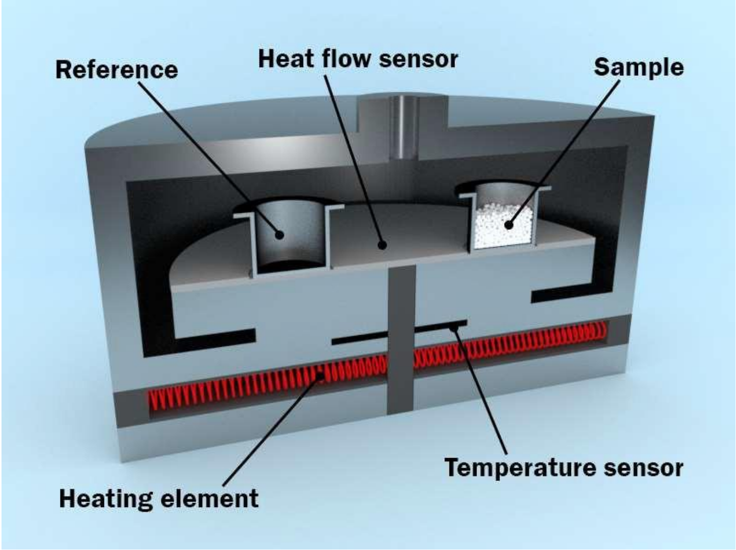
Differential Scanning Calorimetry (e.g., $T_{g'}$)

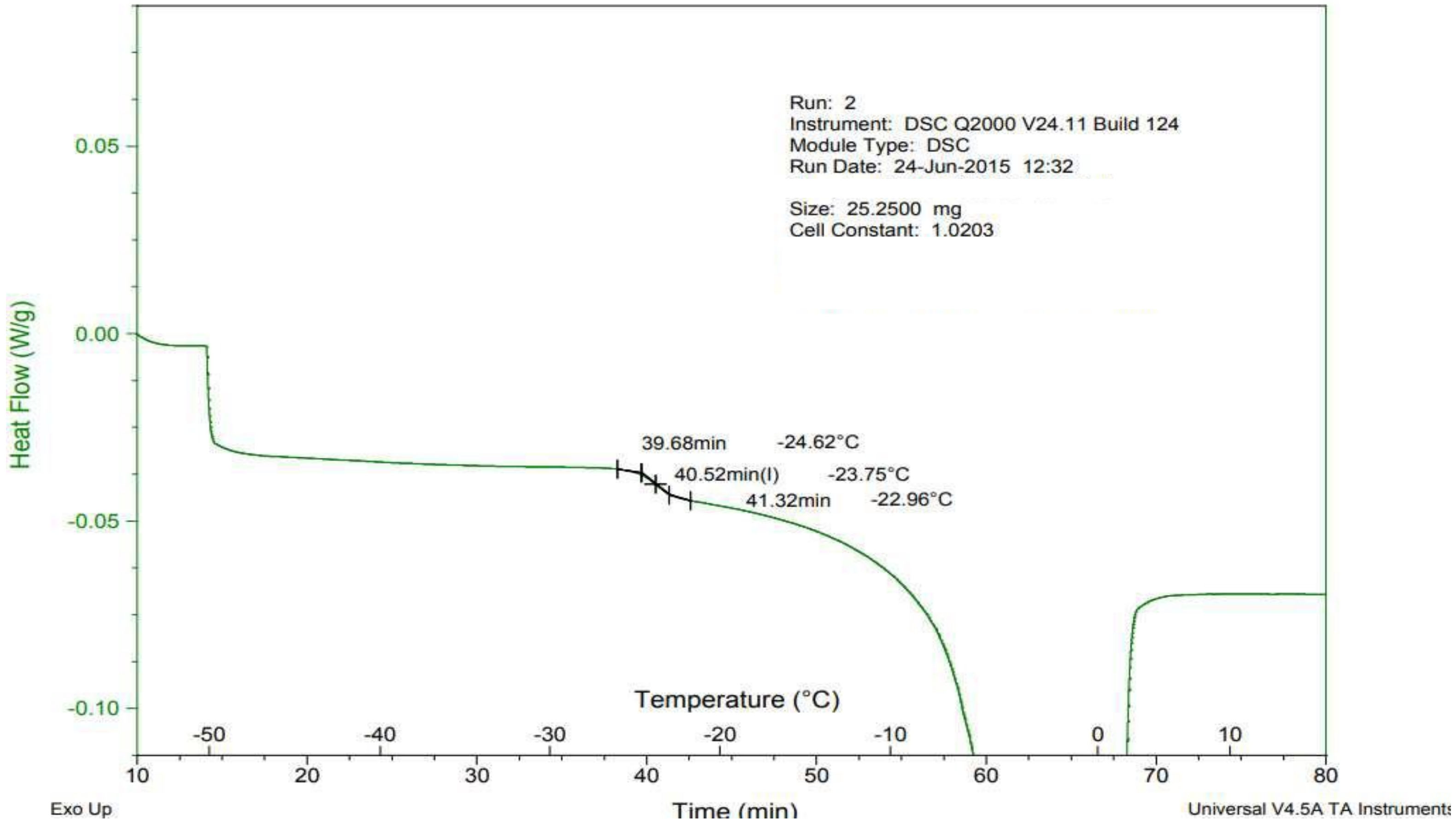




- Thermal analysis to detect physical transformation such as phase transitions (e.g. glass transition temperature $T_{g'}/T_g$, crystallization/melting point T_{eut} …)
- Measurement of the difference in the amount of heat required to increase the temperature of a sample compared to a reference with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

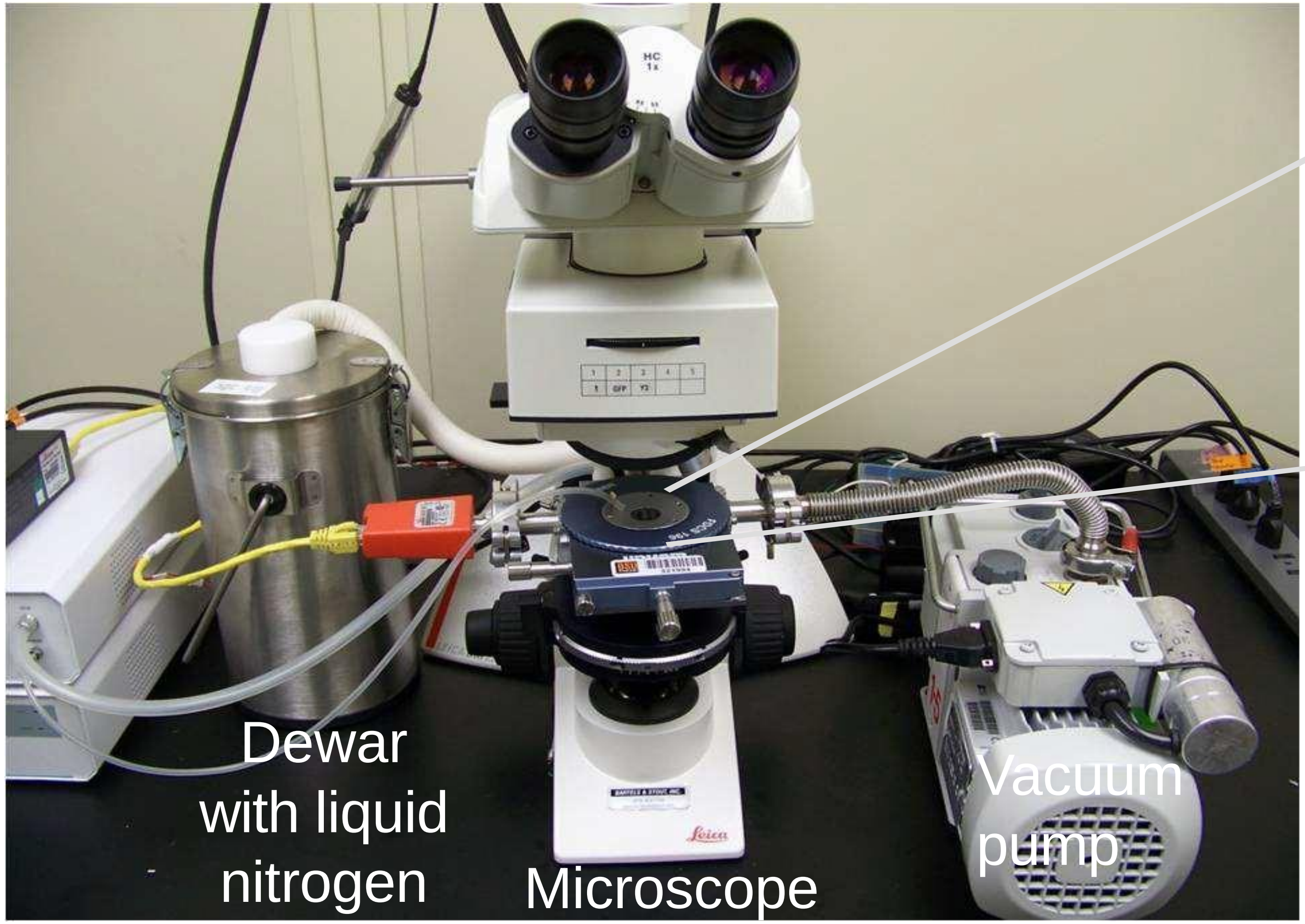
Differential Scanning Calorimetry (e.g., $T_{g'}$)

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



Literature recommendation for design of DSC mesaurements: Pansare SK, Patel SM. Practical Considerations for Determination of Glass Transition Temperature of a Maximally Freeze Concentrated Solution. AAPS PharmSciTech. 2016;17(4):805–19.

Freeze drying microscopy ($T_{collapse}$)

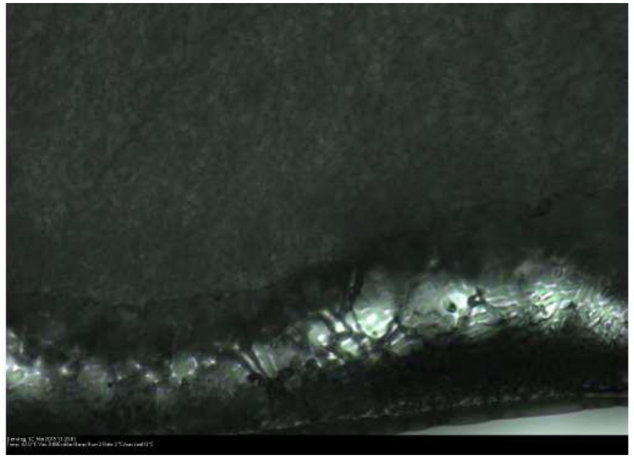


Cryostage

Freeze drying microscopy ($T_{collapse}$)

(Intact) frozen sample

Onset of collapse



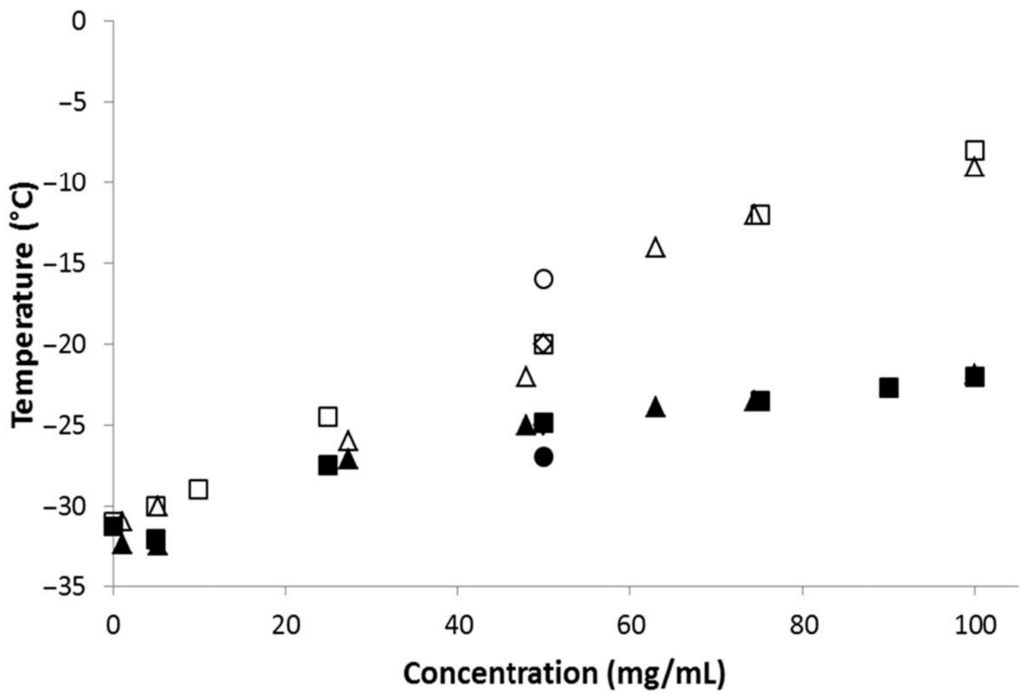
Complete collapse

→ $T_{g'} < T_{collapse}$!!

<u>Rule of thumb:</u> $T_{g'}$ ~2 °C lower than T_c (low protein conc., see side note)

For visualization: https://www.youtube.com/watch?v=SqM69VQboCI

Side note: differences in T_g‘ and T_c in dependence of protein concentration



Glass transition temperature of the maximally freeze-concentrated solution and T_c as a function of protein concentration. Values for mAb A (IgG1) are denoted by triangles, mAb B (IgG1) by circles, mAb C (IgG4) by squares, and Pro X (fusion protein, 90kDa) by diamonds. Closed symbols: $T_{g'}$; open symbols: T_c.

- The higher the protein concentraton, the larger the deviation between T_c and T_g‘ (up to ~14 °C at high protein conc.)
- Thus, Freeze drying microscopy analysis always more accurate than DSC only

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Residual moisture – Water content

Gravimetric analysis

- Loss of mass in drying cabinet (TGA) or IR balance
- Targets any volatile component
- Destructive
- Loss on drying (LOD) may be challenging for lyos (weight of dry product & expected water content low)

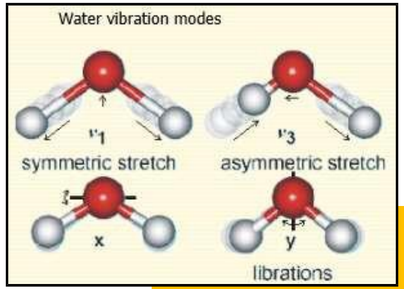
Karl-Fischer titration

- Quantitative water determination by titration

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

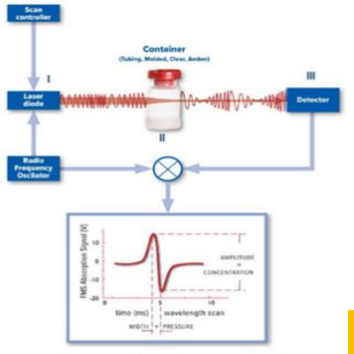
$$H_2O + I_2 + (RNH)SO_3CH_3 + 2\ RN \rightarrow (RNH)SO_4CH_3 + 2\ (RNH)I$$

- 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) including cross-validation with KF-results



Schematic of FMS for laser-based headspace 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!)

More details in the excellent guest presentation by Derek Duncan

Karl-Fischer Titration

Volumetric 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).

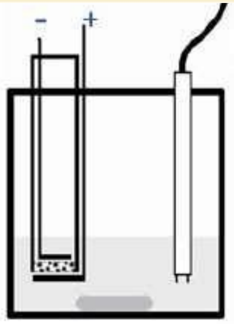
Volumetric Karl Fischer Titration
Iodine is added by a burette during titration.
Suitable for samples where water is present as a major component: **100 ppm - 100%**

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

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

Redox reaction

Coulometric Karl Fischer Analysis

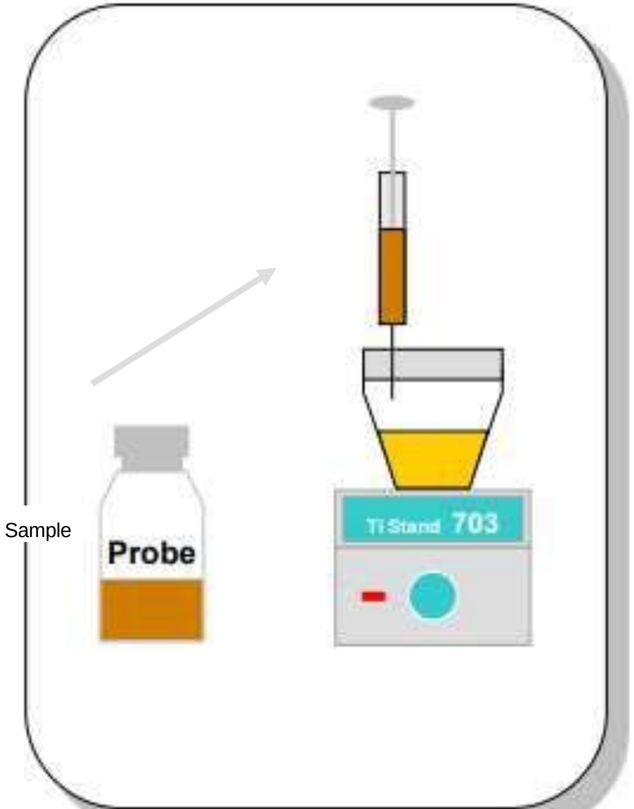


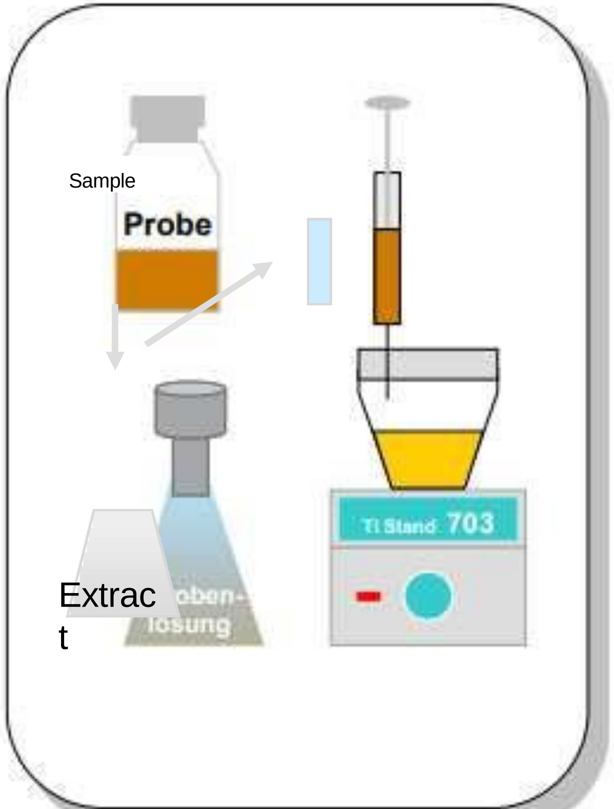
Coulometric Karl Fischer Analysis
Iodine is generated electrochemically during titration.
Suitable for samples where water is present in trace amounts: **1 ppm - 5%**

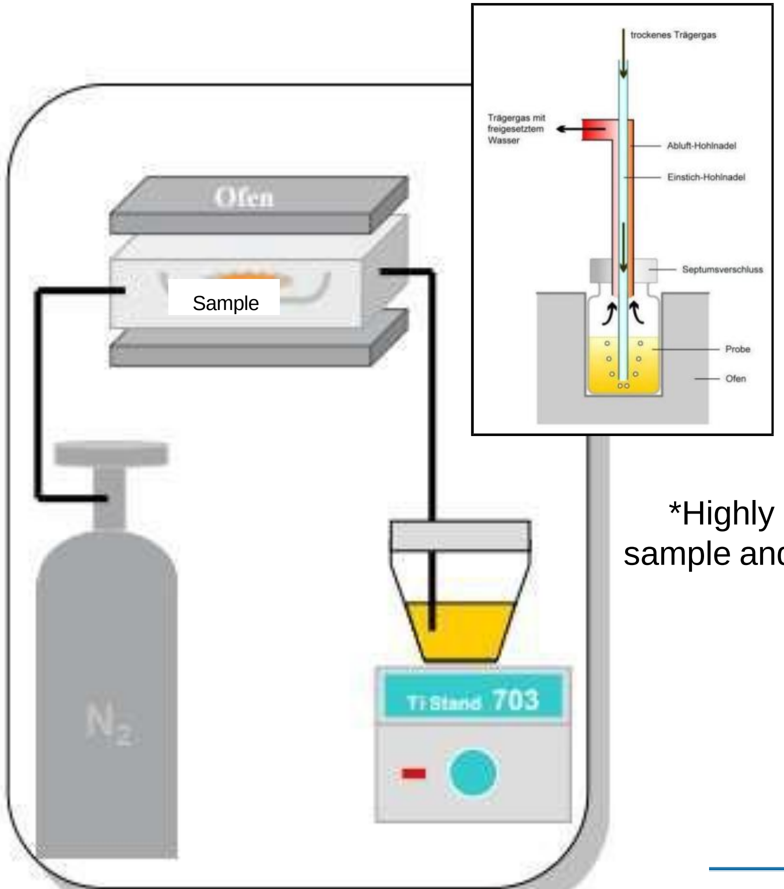
- The working medium consists of the components sulfur dioxide, alcohol, and organic base or/and water free vehicle.
- Two electrodes are needed: One for Iodine generation (anode), and one for potentiometric end-point detection via the indicator electrode (free I_2/I- redox couple).

Karl-Fischer Titration

Direct Titration	Liquid Extraction	Evaporation*

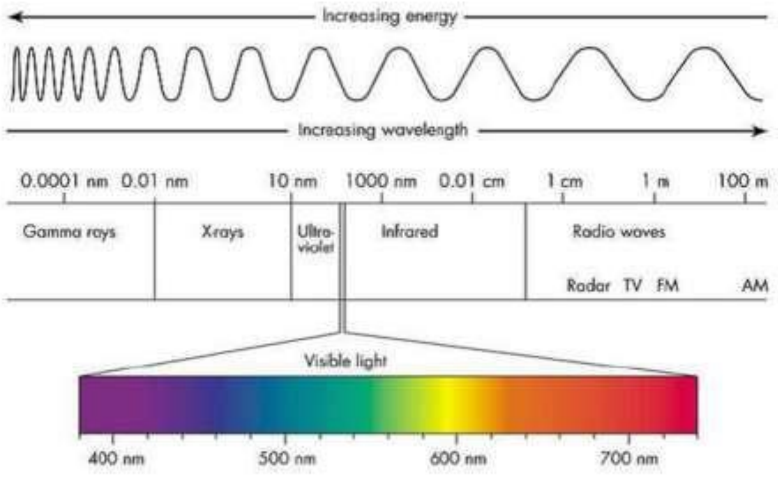


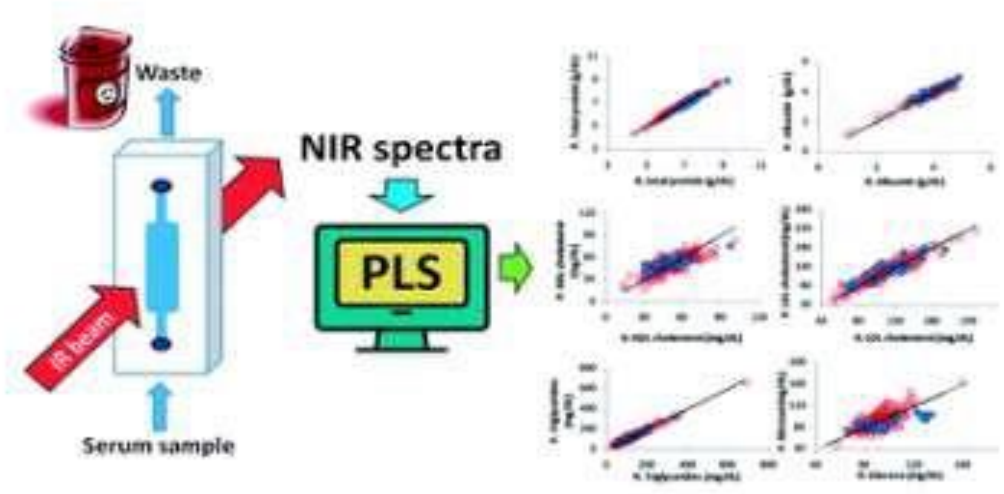


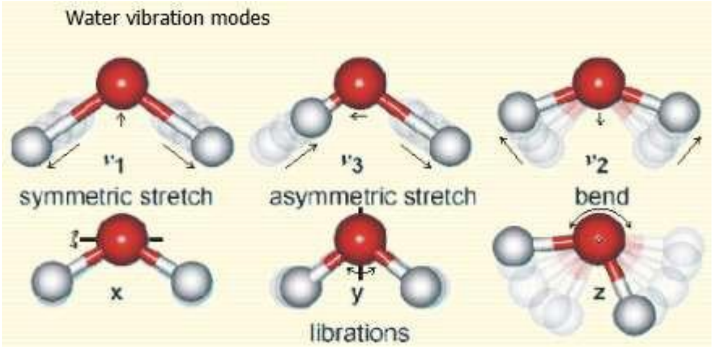


*Highly dependent on the sample and its heat sensitivity.

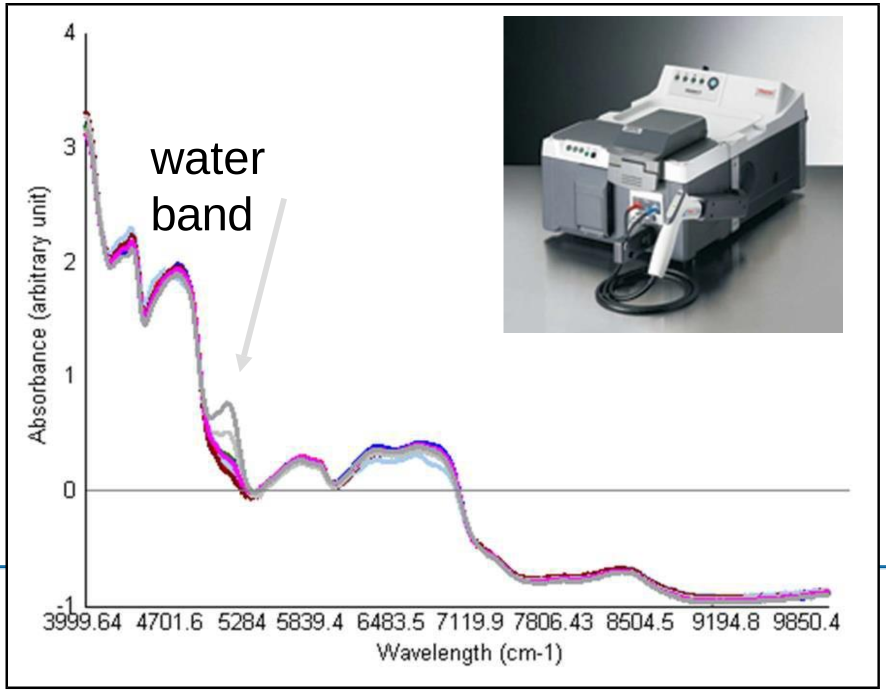
Residual moisture - NIR



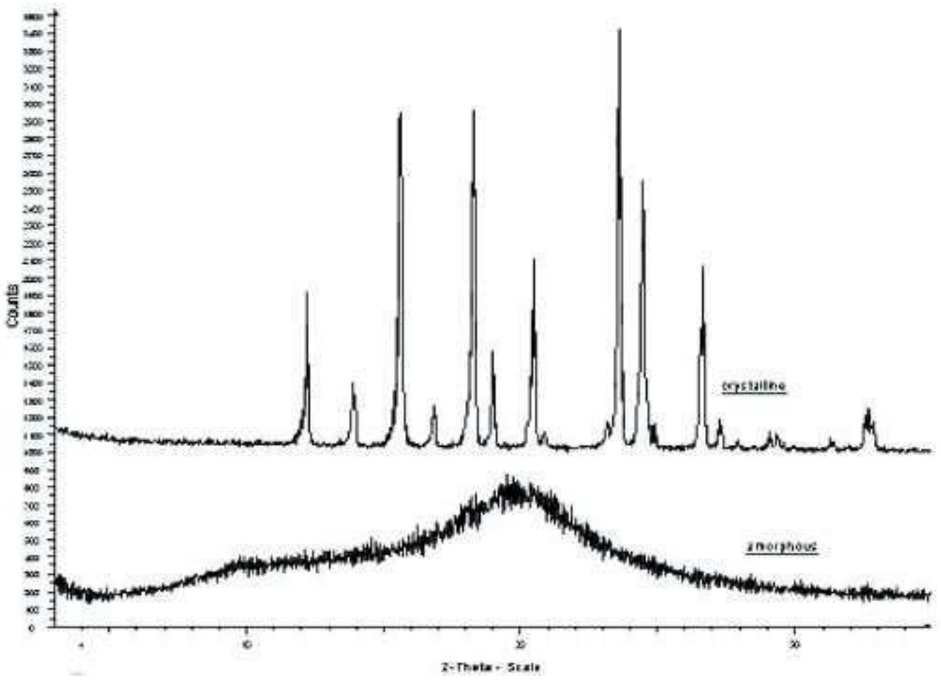




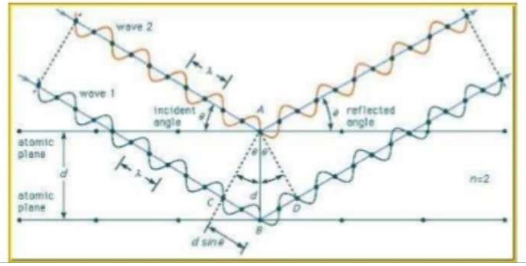
- Molecule vibrations (overtone and combinations)
- Near infrared: ~760–2500 nm or 13.000–4.000 cm−1



Xray powder diffraction - Morphology



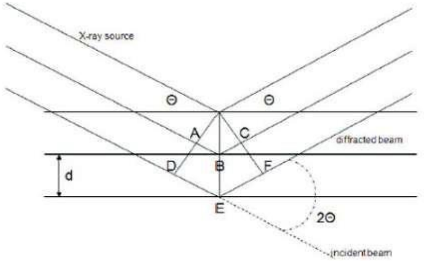
- A crystalline powder contains many small crystallites, ideally randomly oriented
- Diffraction occurs when crystallites are oriented such that specific atomic planes are in the correct relationship with the incoming x-rays



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

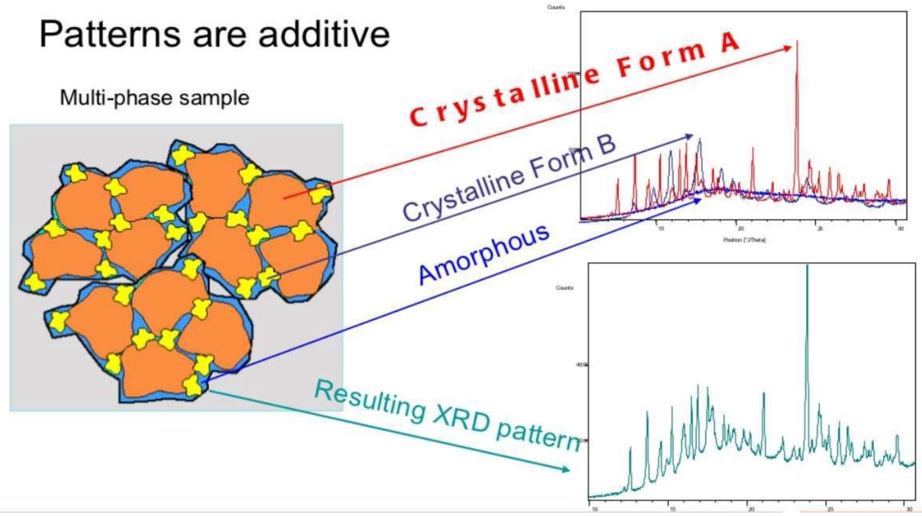
Constructive interference is detected when the path-length difference is equal to an integer number of wavelengths

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



Mixture analysis

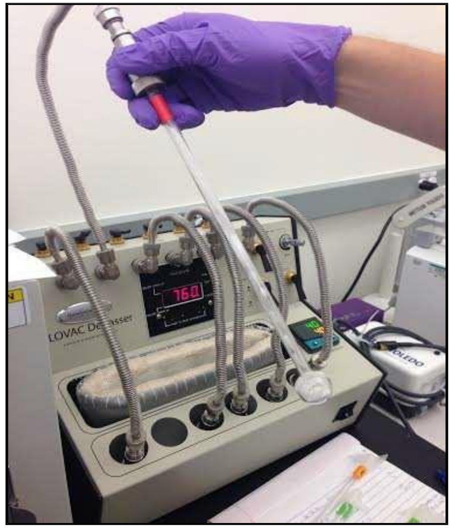
Patterns are additive



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 accessible pores.



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

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Cake appearance: visual inspection

Reprinted from: „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.
2017. J Pharm Sci. 106(7) Copyright [2017] © Elsevier B.V., its licensors, and contributors

Cosmetic defects versus impact on product quality?

Intact cake

Shrinkage

Light collapse / melt-back

severe collapse / melt-back

complete collapse collapse/melt-back

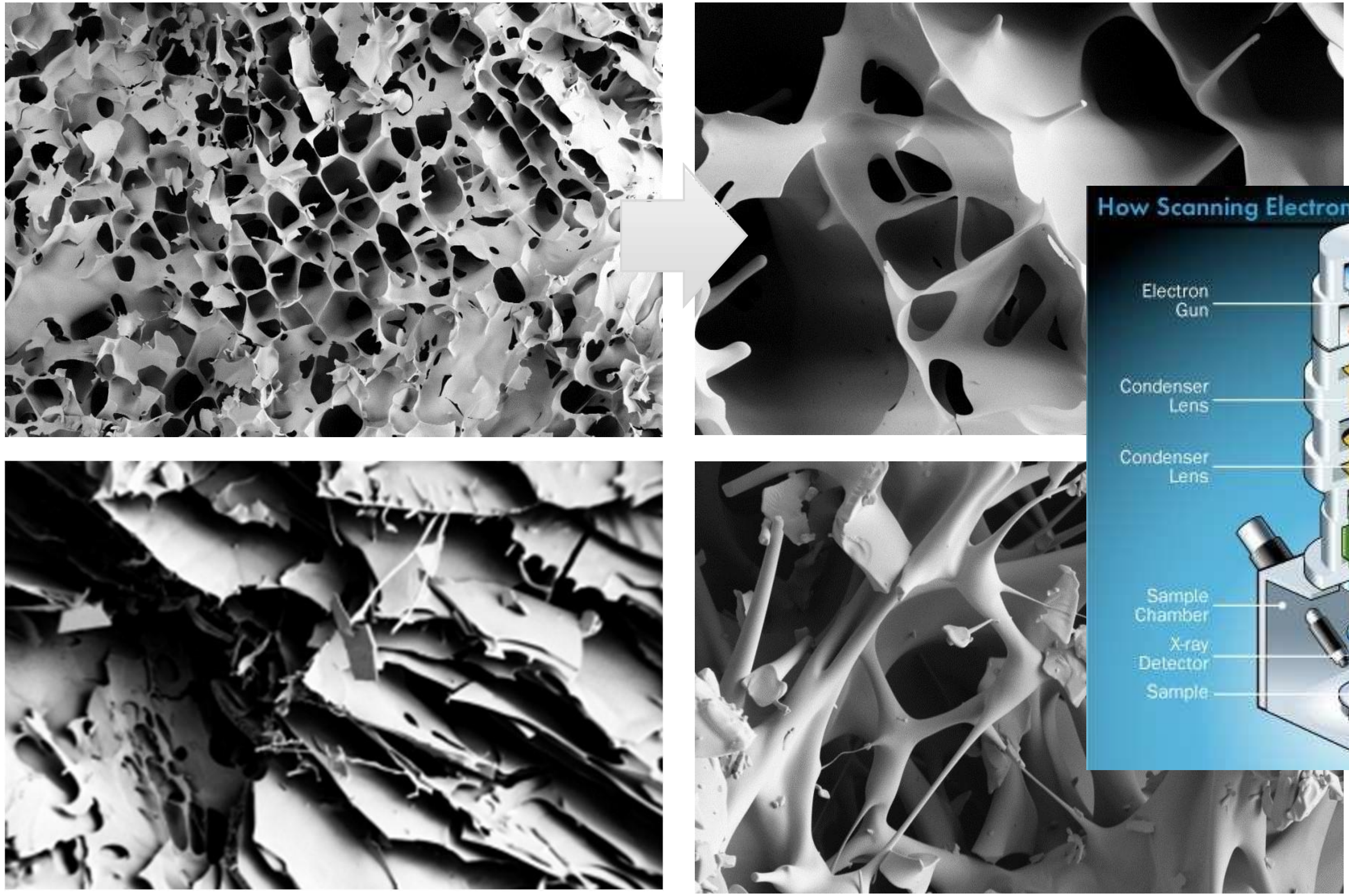
crack

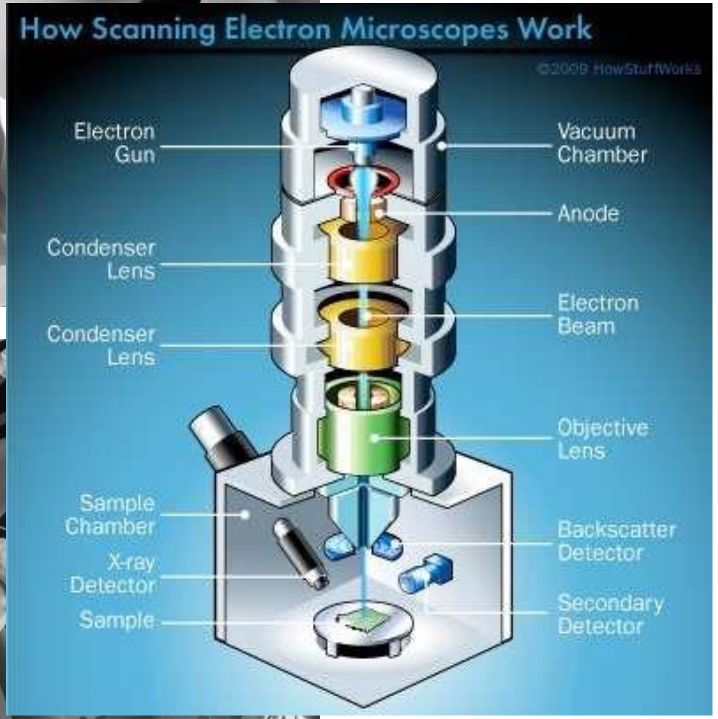
dents

(minor) splashing

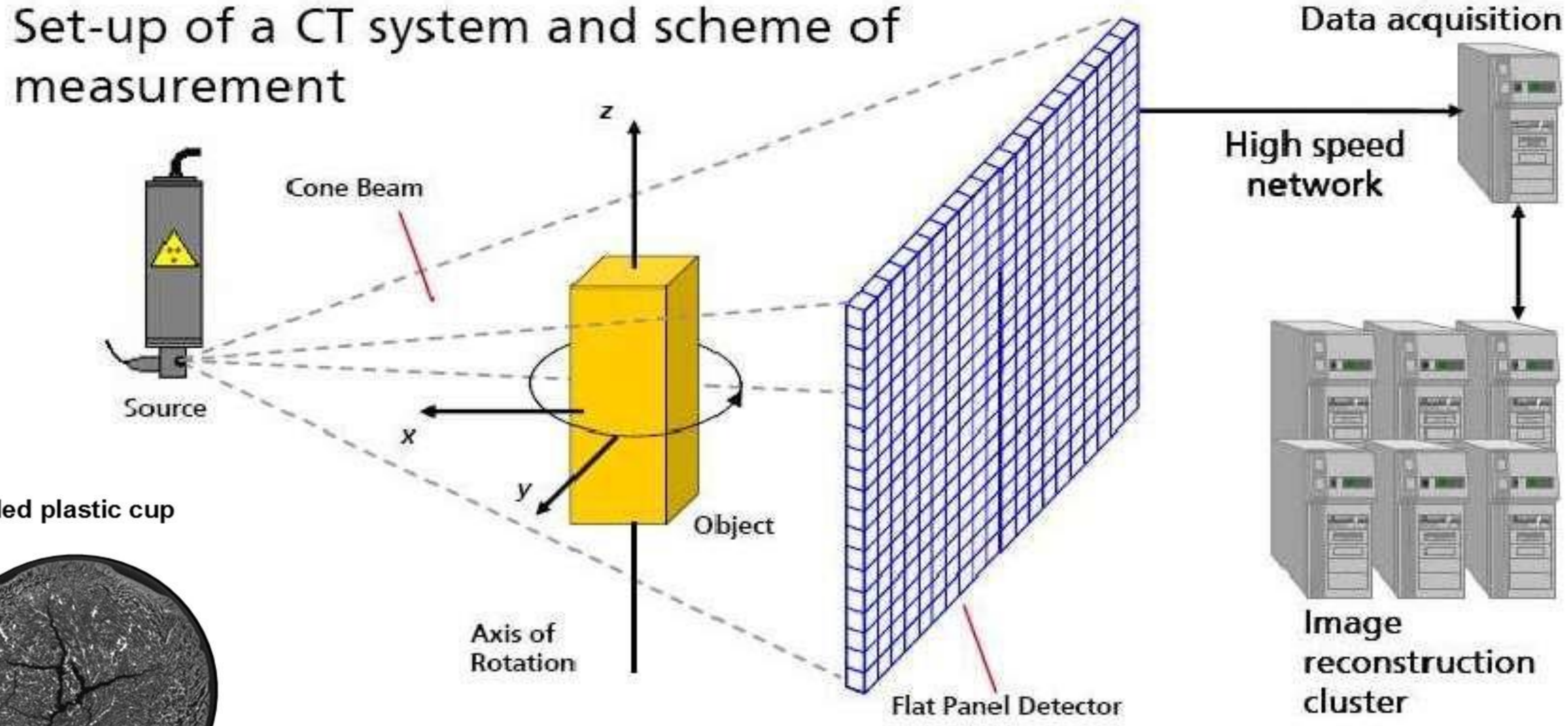
fogging

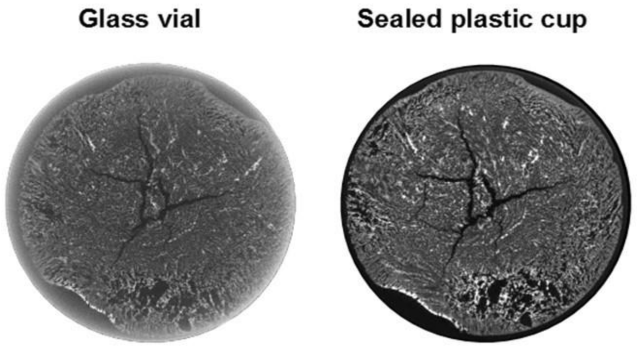
Cake appearance: Scanning electron microscopy (SEM)





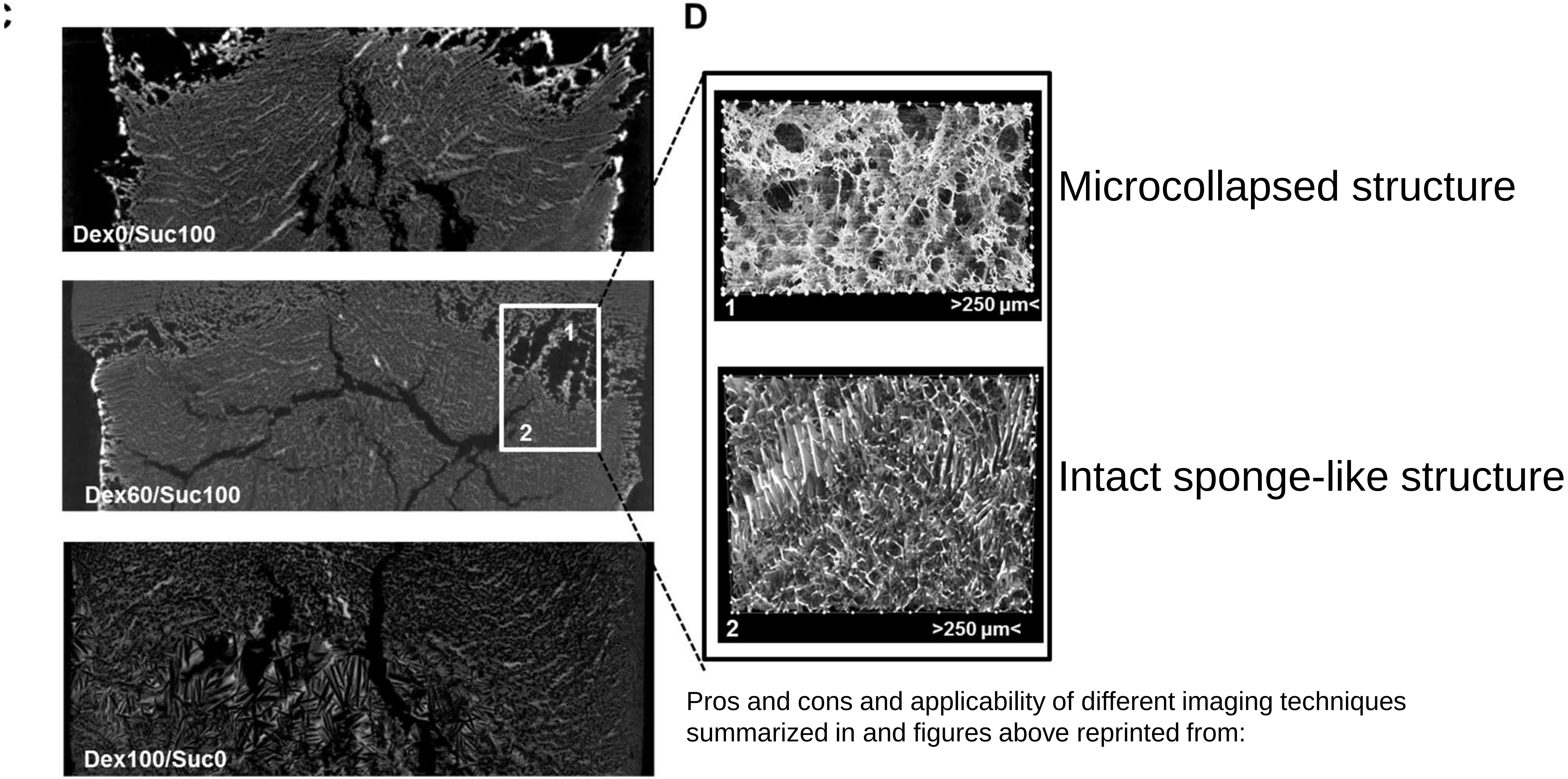
Cake apperance: (X-ray) Micro-computed tomography (µCT)





- 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.

Cake apperance: Micro-computed tomography (µCT)



Pros and cons and applicability of different imaging techniques summarized in and figures above reprinted from:

"Imaging techniques to characterize cake appearance of freeze-dried Products". Häuser et al., 2018, J Pharm Sci. 107(11). Copyright [2018] © Elsevier B.V., its licensors, and contributors

Determination of the reconstitution volume

Note: Reconstitution volume ≠ Filling volume (solid content needs to be considered)

Two practical approaches:

1. **Measuring the loss on drying during freeze-drying** (solution density unknown)
   - Weigh selected filled and semi-stoppered vials before and after freeze-drying
   - Mass difference can be accounted to water loss on drying
   - Mass loss can be converted to the reconstitution volume by division by density of water

2. **Calculate the total amount of water that could be lost on drying** (solution density known)
   - Determine the density of your formulated solution to be freeze-dried
   - Calculate the exact total solid content based on composition
   - Calculate the theoretically filled mass (by multiplication with formulation density)
   - Substract the total solid content from the theoretically filled mass
   - Calculated mass difference can be converted to the reconstitution volume by division by density of water

Reconstitution time

→ Water ideally flows along the side wall

→ Avoid foaming if samples contain surfactants

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

Literature recommendation:
„Key factors governing the reconstitution time of high concentration lyophilized protein formulations” Kulkarni et al., Eur J Pharm Biopharm. 2021. Vol. 165
“Reconstitution of Highly Concentrated Lyophilized Proteins: Part 1 Amorphous Formulations.” Sane et al. J Pharm Sci. 2020. 109(5).
“Reconstitution Time for Highly Concentrated Lyophilized Proteins: Role of Formulation and Protein” Kulkarni et al. J Pharm Sci. 2020. 109(10)