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Theory 9

Analytical characterization

Product attributes for designing lyophilization cycles

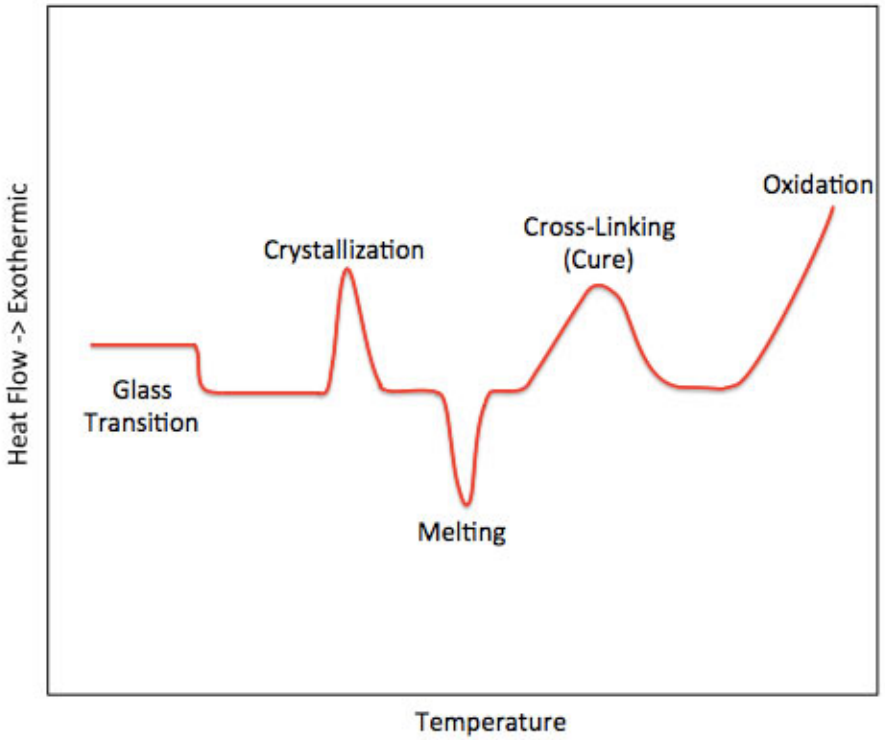
- Differential scanning calorimetry: $T_{g'}$, T_g, T_{eut}
- Freeze drying microscopy: $T_{collapse}$

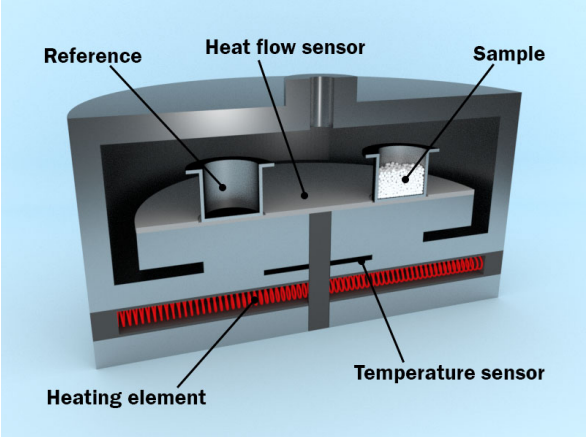
Solid state characterization after lyophilization

- 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

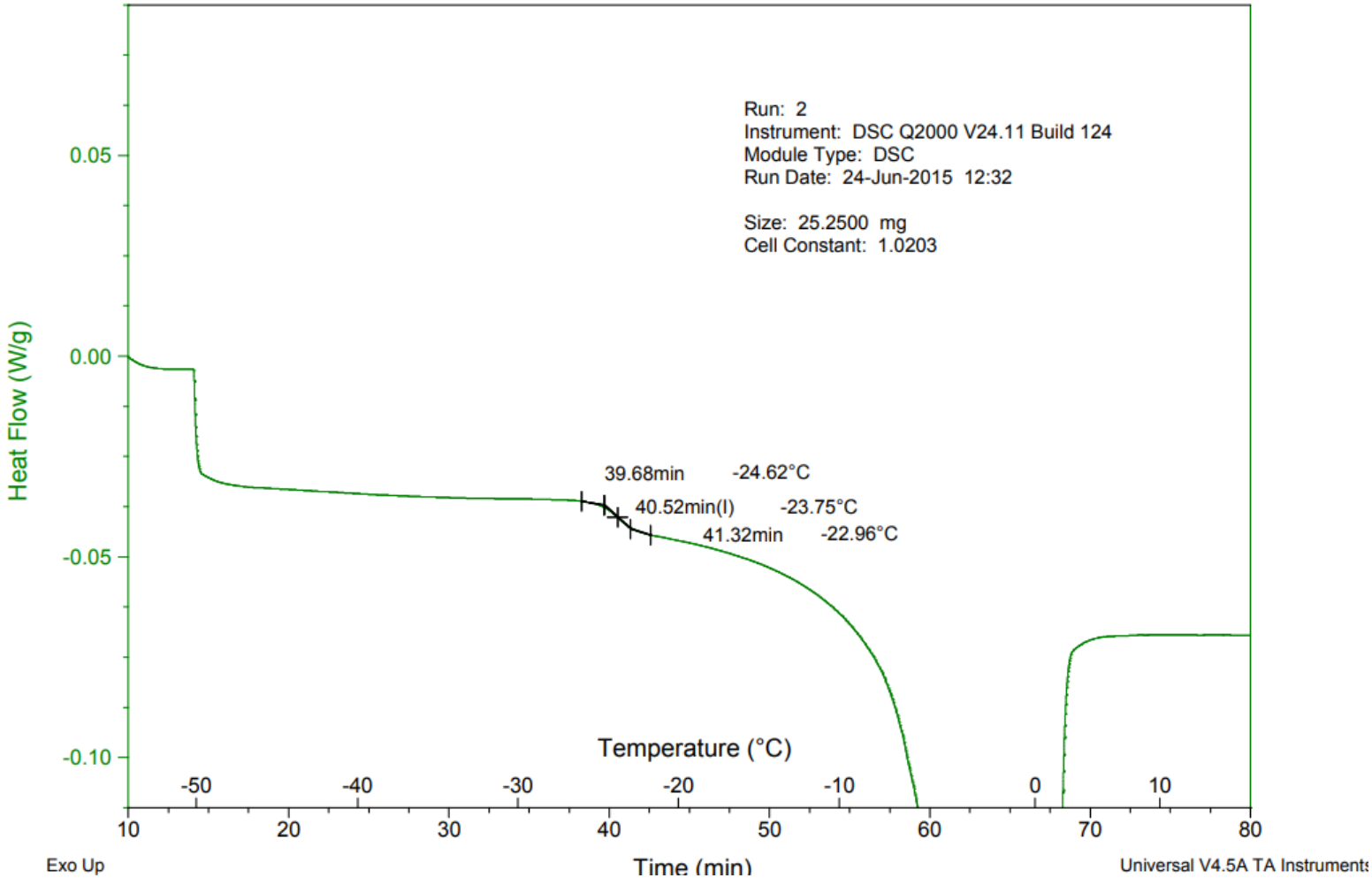
Differential Scanning Calorimetry (e.g. $T_{g'}$)



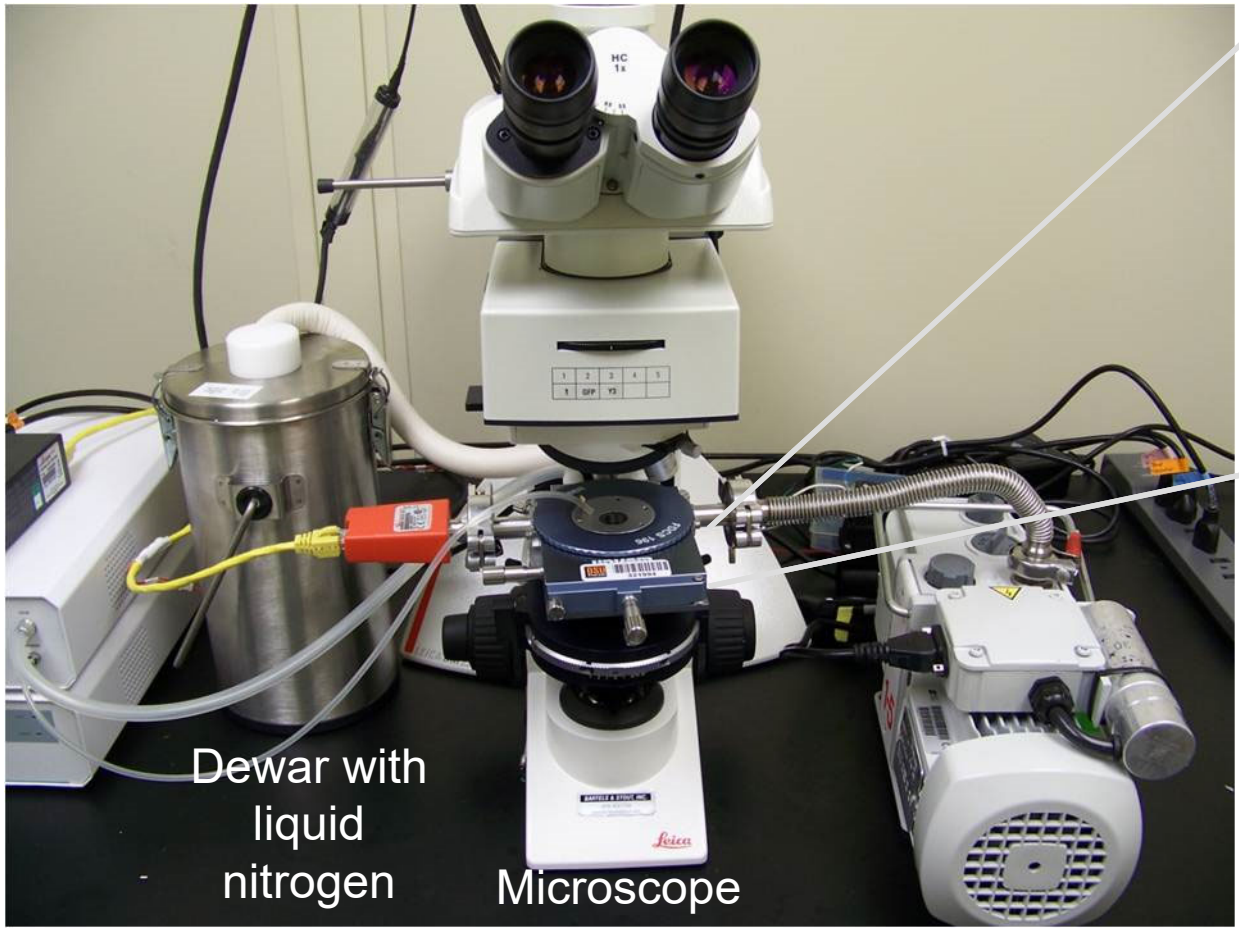


- Thermal analysis to detect physical transformation such as phase transitions (e.g. glass transition temperature $T_{g'}/T_g$, crystallization/melting point T_{eut} …)
- Measurement of the difference in the amount of heat required to increase the temperature of a sample compared to a reference with 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. $T_{g'}$)



Freeze drying microscopy ($T_{collapse}$)

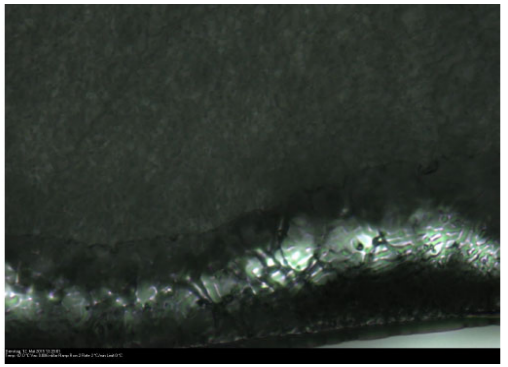


Cryostage

Freeze drying microscopy ($T_{collapse}$)

(Intact) frozen sample

Onset of collapse



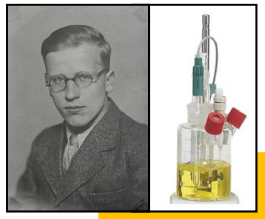
Complete collapse

→ $T_{g'} < T_{collapse}$!!

Residual moisture – Water content

Gravimetric analysis

- Loss of mass in drying cabinet or IR
- Targets any volatile components
- Destructive
- Does not account for ‘hidden’ water



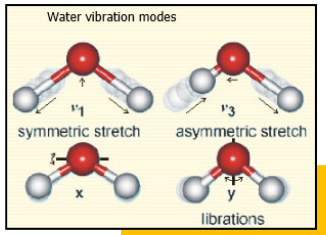
Karl-Fischer

- 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)

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).

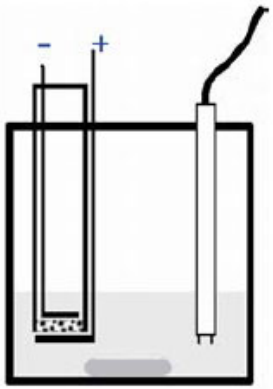
Volumetric Karl Fischer Titration

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



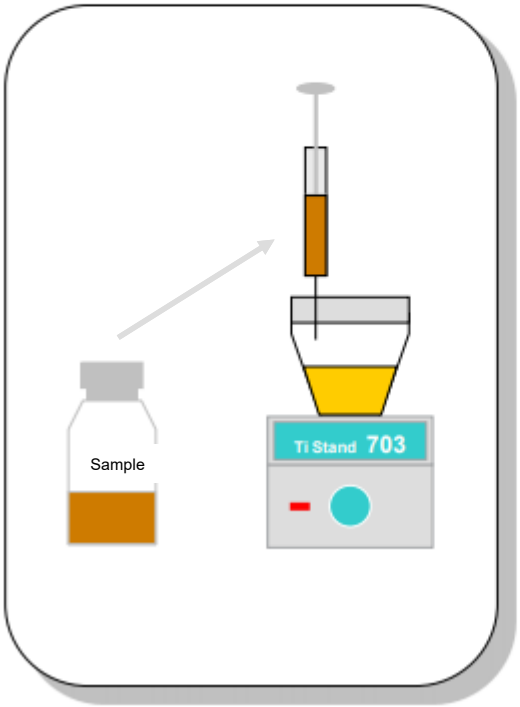
Coulometric Karl Fischer Analysis

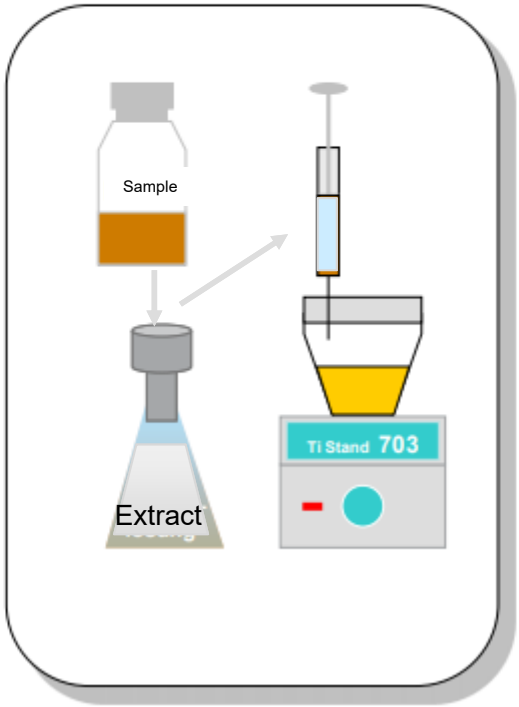
Iodine is generated electrochemically during titration.
Suitable for samples where water is present in trace amounts: **1 ppm - 5%**

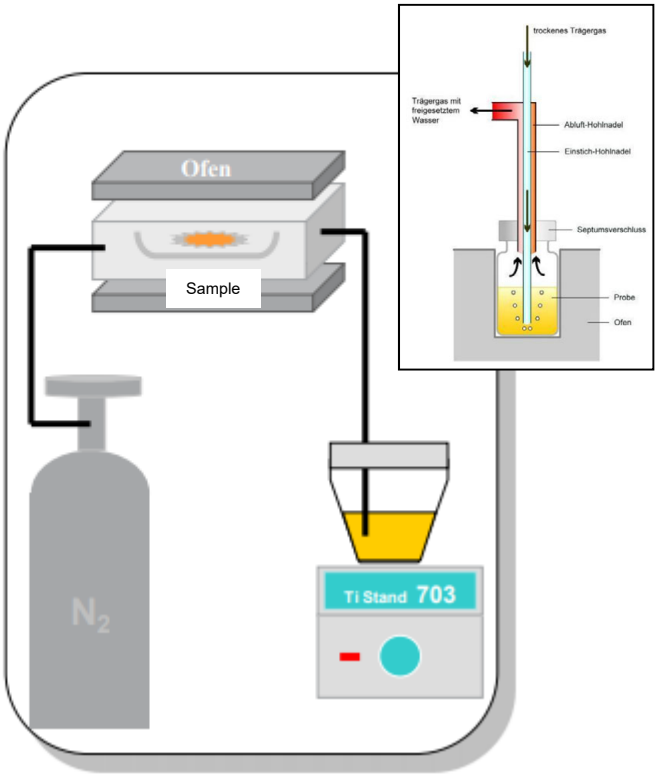
- The working medium consists of the components sulfur dioxide, alcohol, and organic base or/and water free vehicle.
- Two electrodes are needed: One for Iodine generation (anode), and one for potentiometric end-point detection via the indicator electrode (free I_2/I- redox couple).

Karl-Fischer Titration

Direct Titration	Liquid Extraction	Evaporation

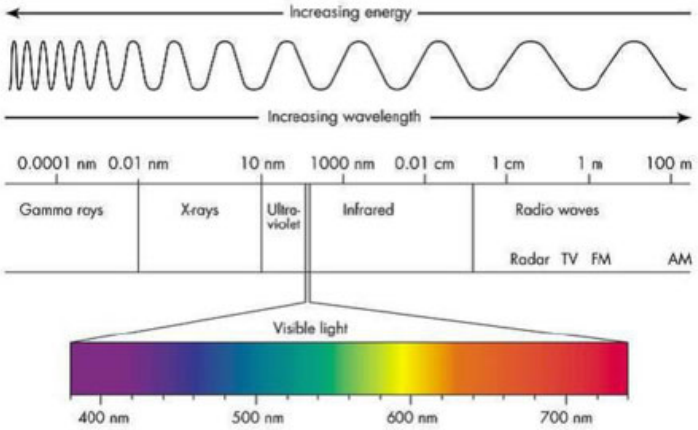


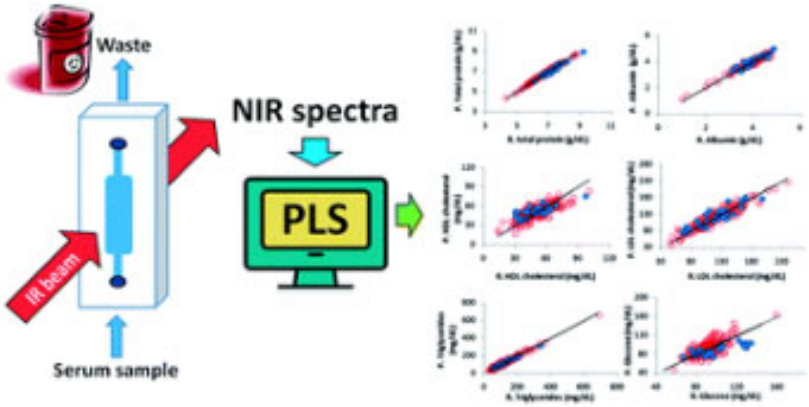


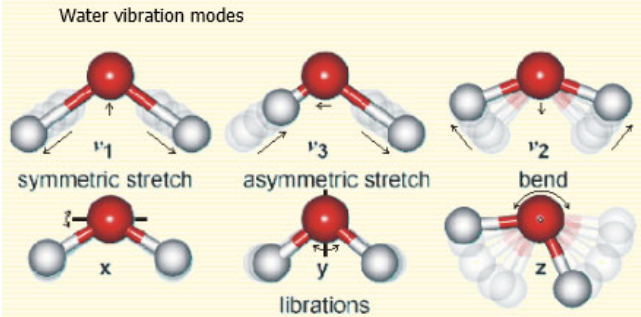


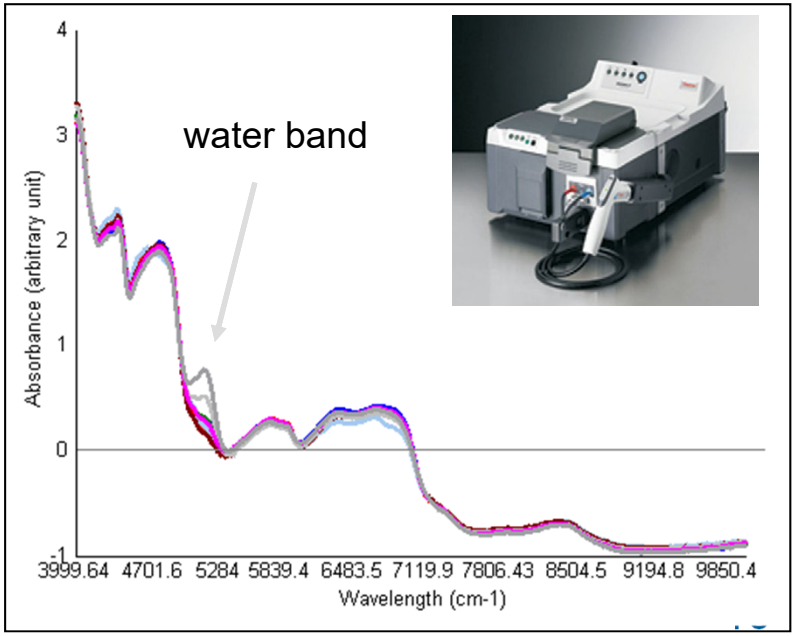
Highly dependent on the sample and its heat sensitivity.

Residual moisture - NIR









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

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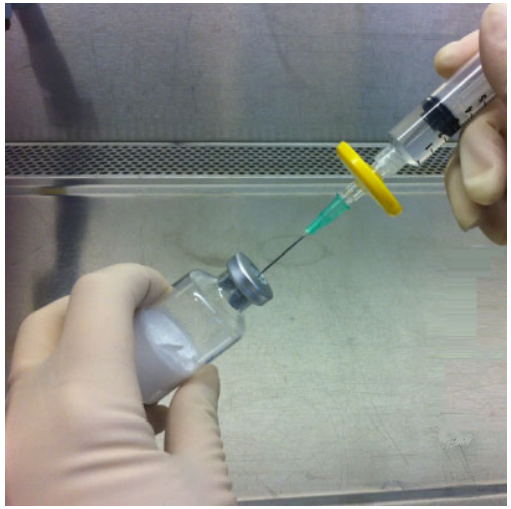
Other quality attributes of active compound

Reconstitution time

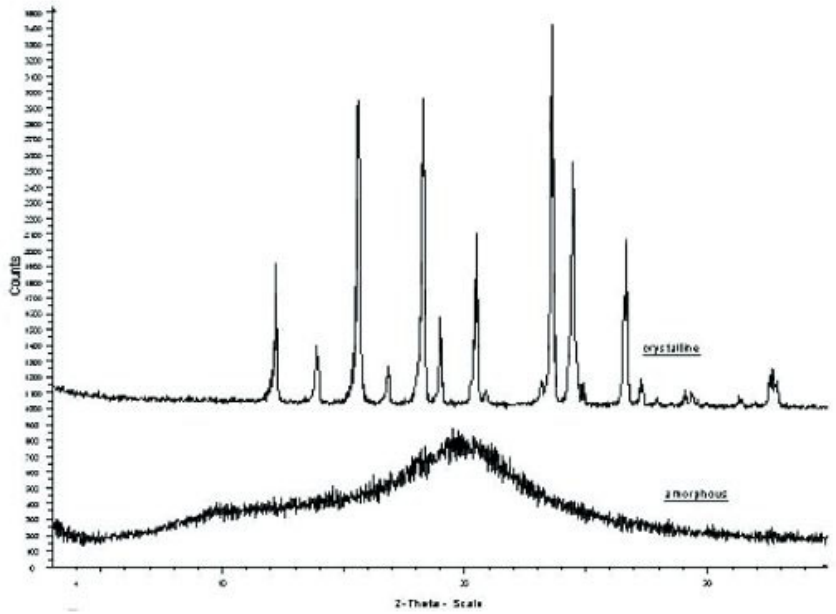


→ Water ideally flows along the side wall

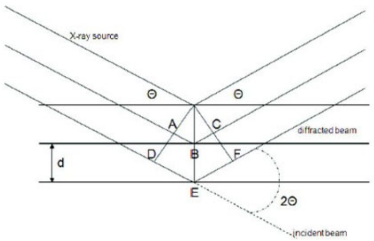
→ Avoid foaming if samples contain surfactants

→ In case of long reconstitution times, shaking systems may be considered

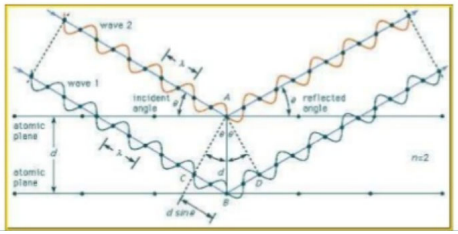
Xray powder diffraction - Morphology



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

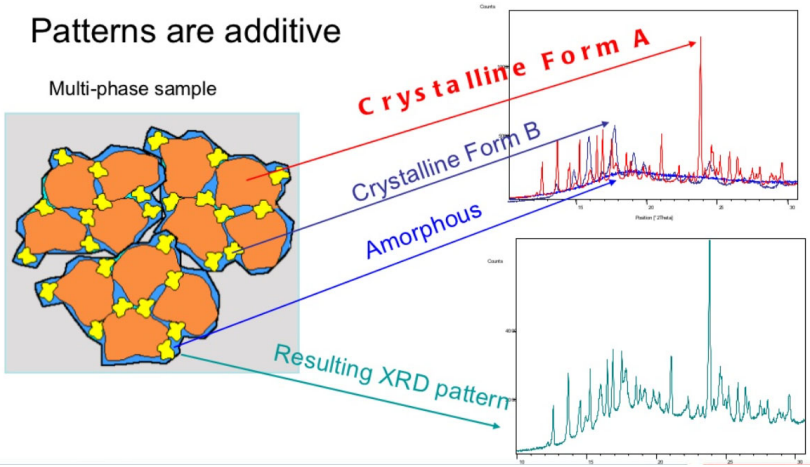


Bragg's law:
$n\lambda=2d\sin\theta$

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

Mixture analysis

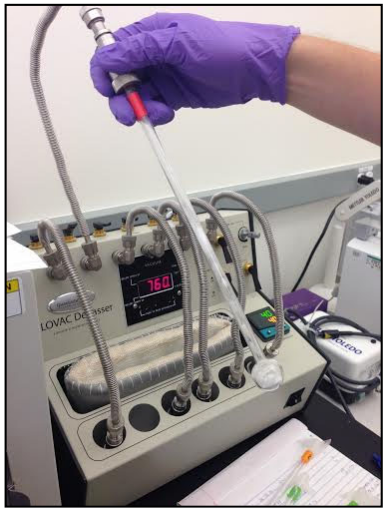
Patterns are additive

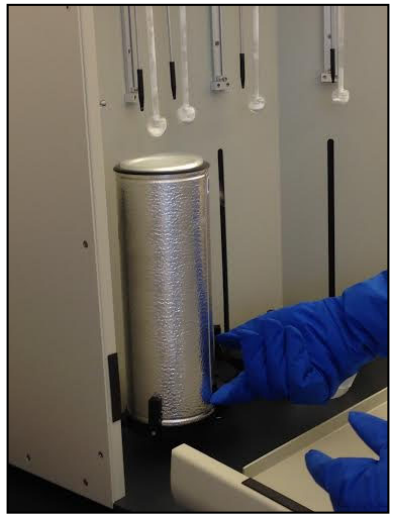


Specific surface area (BET)

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 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.





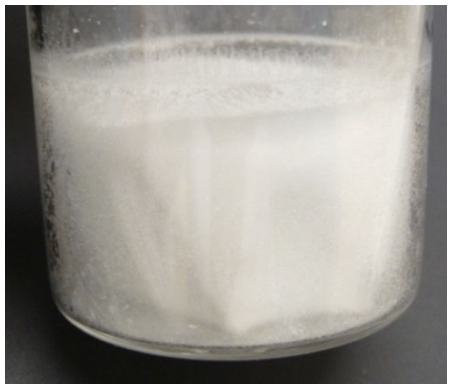
Sample preparation: degasing under vacuum and elevated temperature followed by measurement in liquid N2.

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



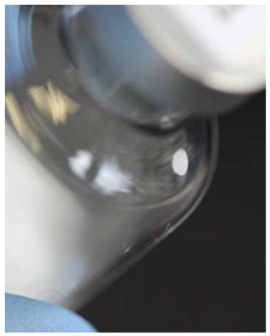
light collapse/melt-back

severe collapse/melt-back

complete collapse/melt-back

crack

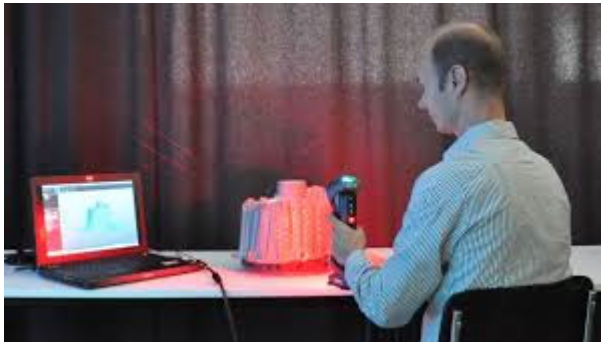
dents



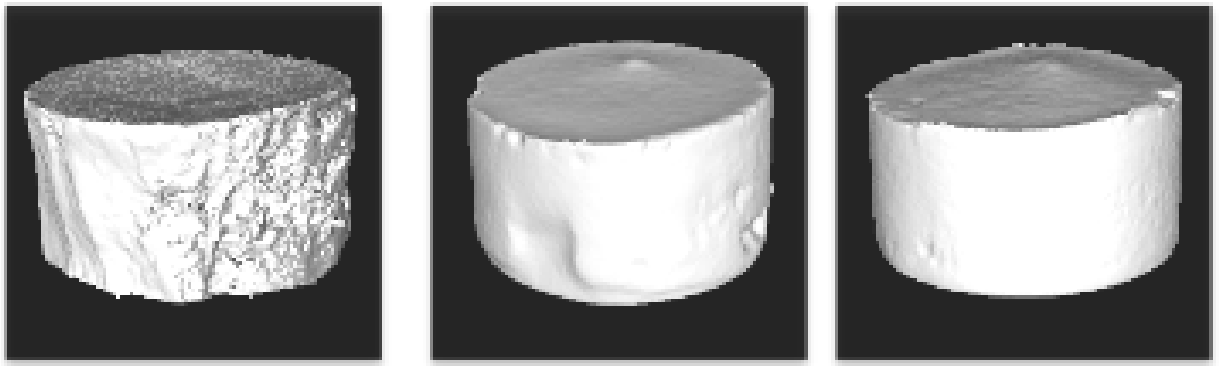
splashing

fogging

3D scanning



Dex0/Suc100 Dex60/Suc40 Dex100/Suc0



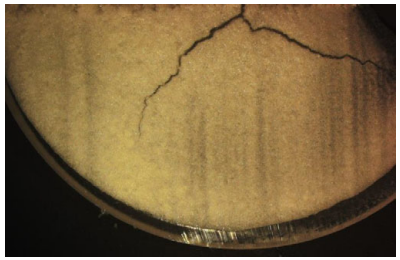
PDMS embedding

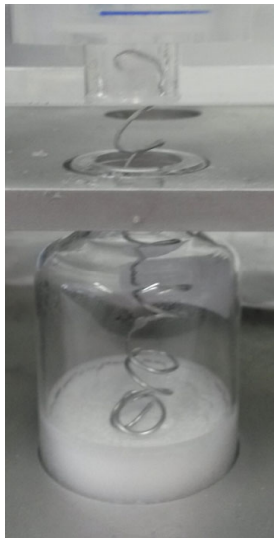
PDA Journal
of Pharmaceutical Science and Technology

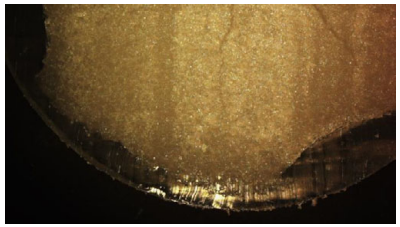
An Improved Method for Visualizing the Morphology of Lyophilized Product Cakes

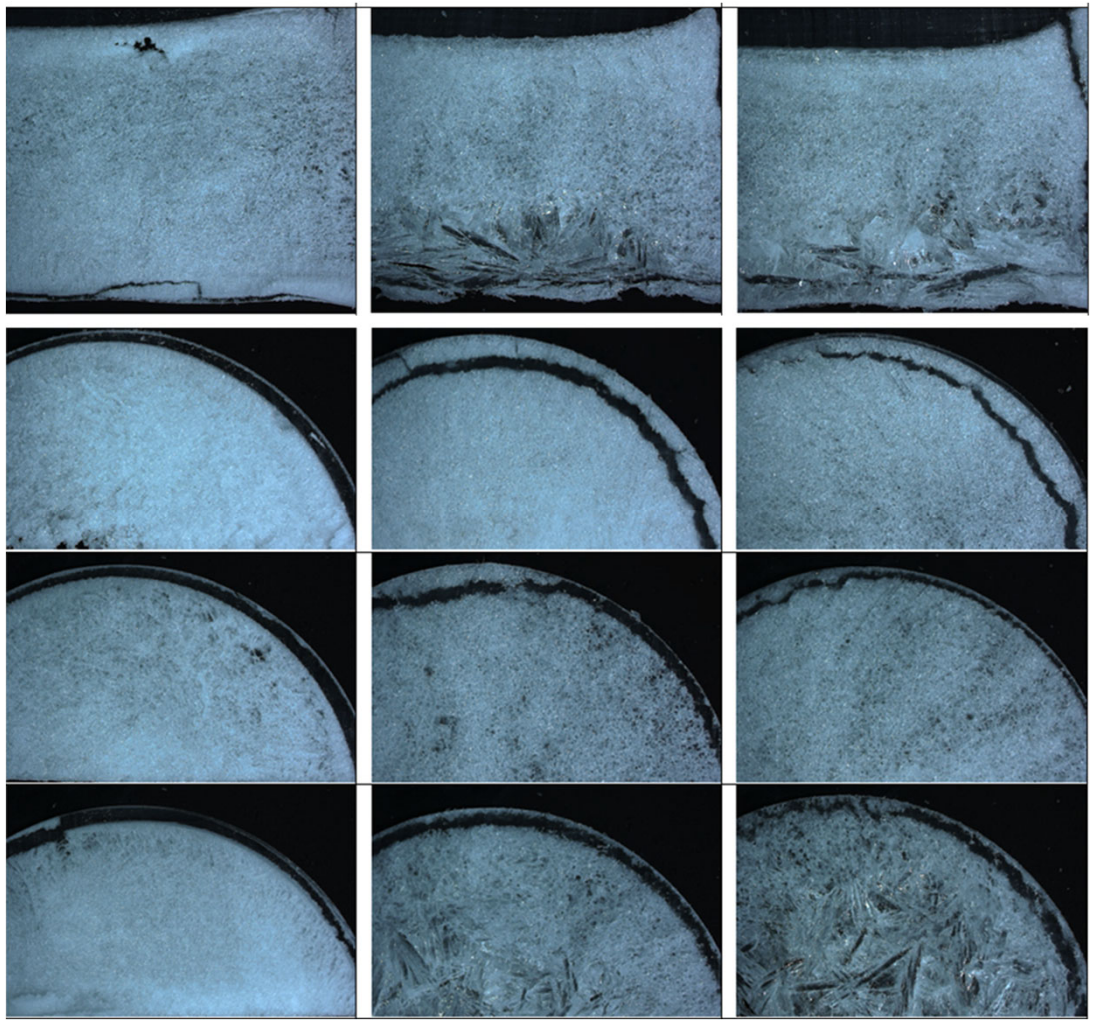
Philippe Lam and Thomas W. Patapoff

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

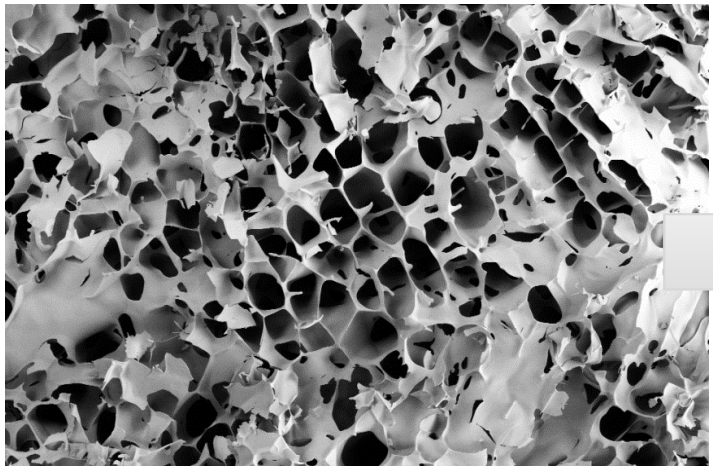


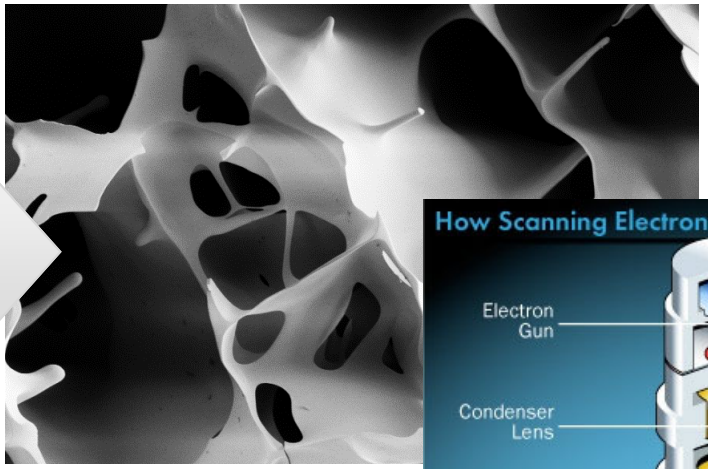


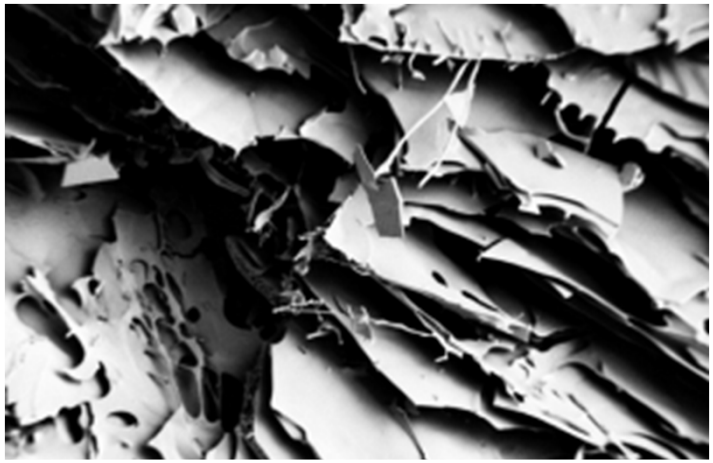


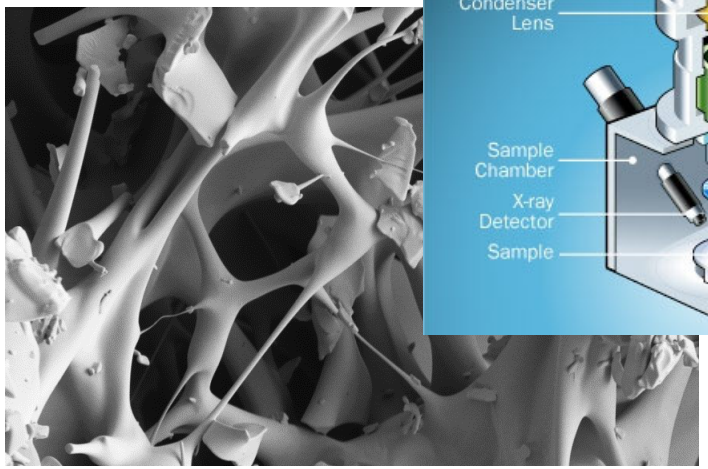


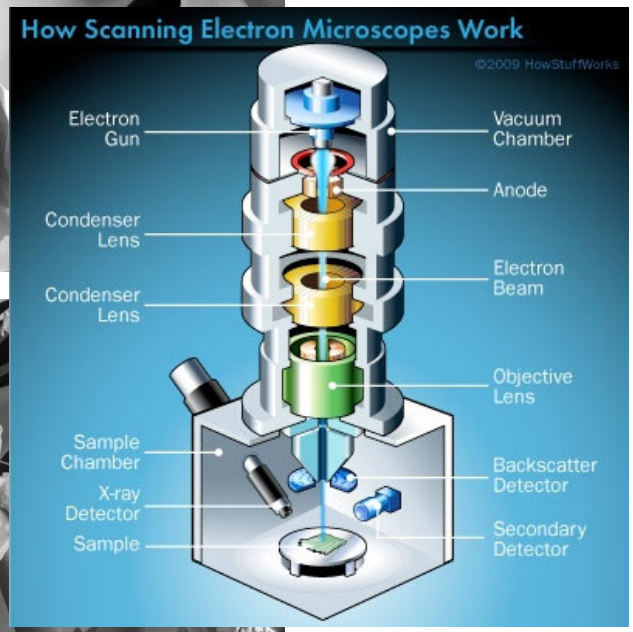
Scanning electron microscopy (SEM)



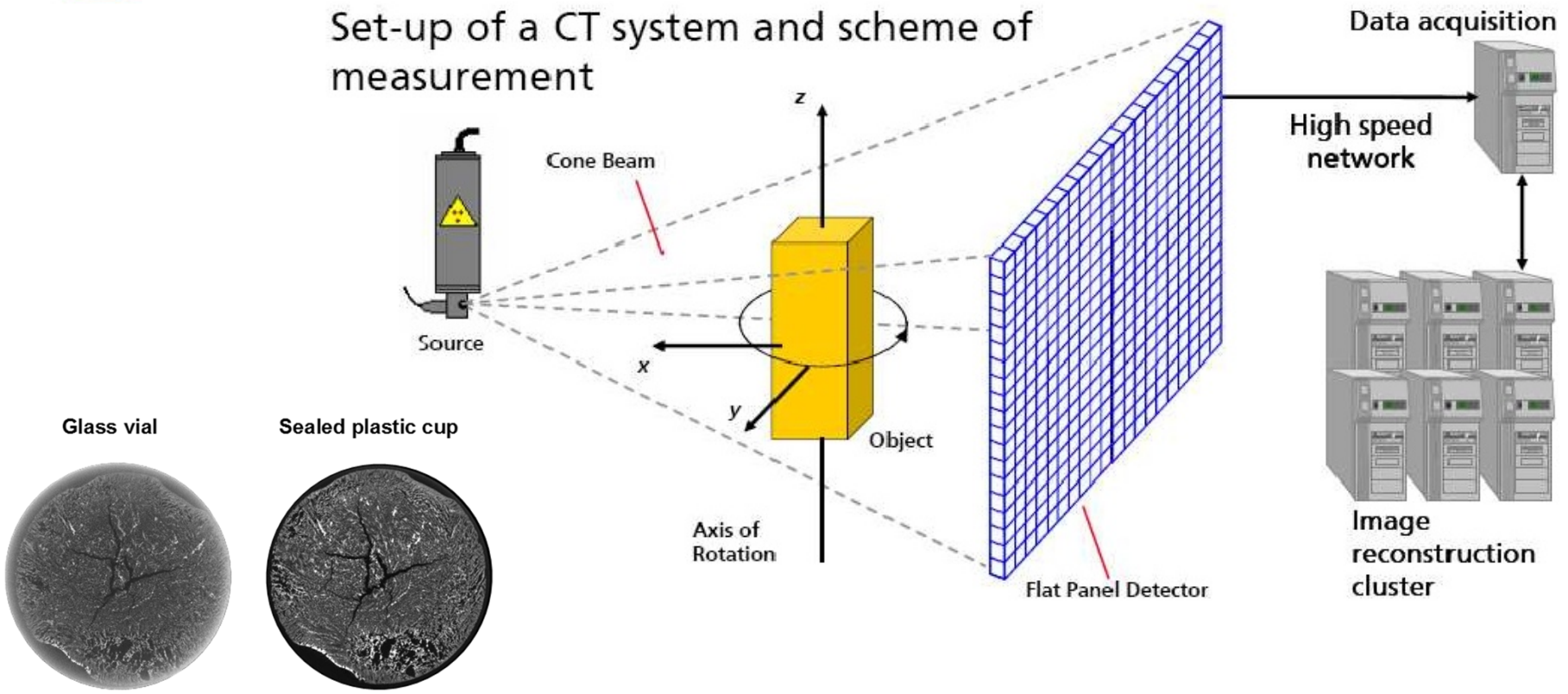








Micro-computated tomography (µCT)



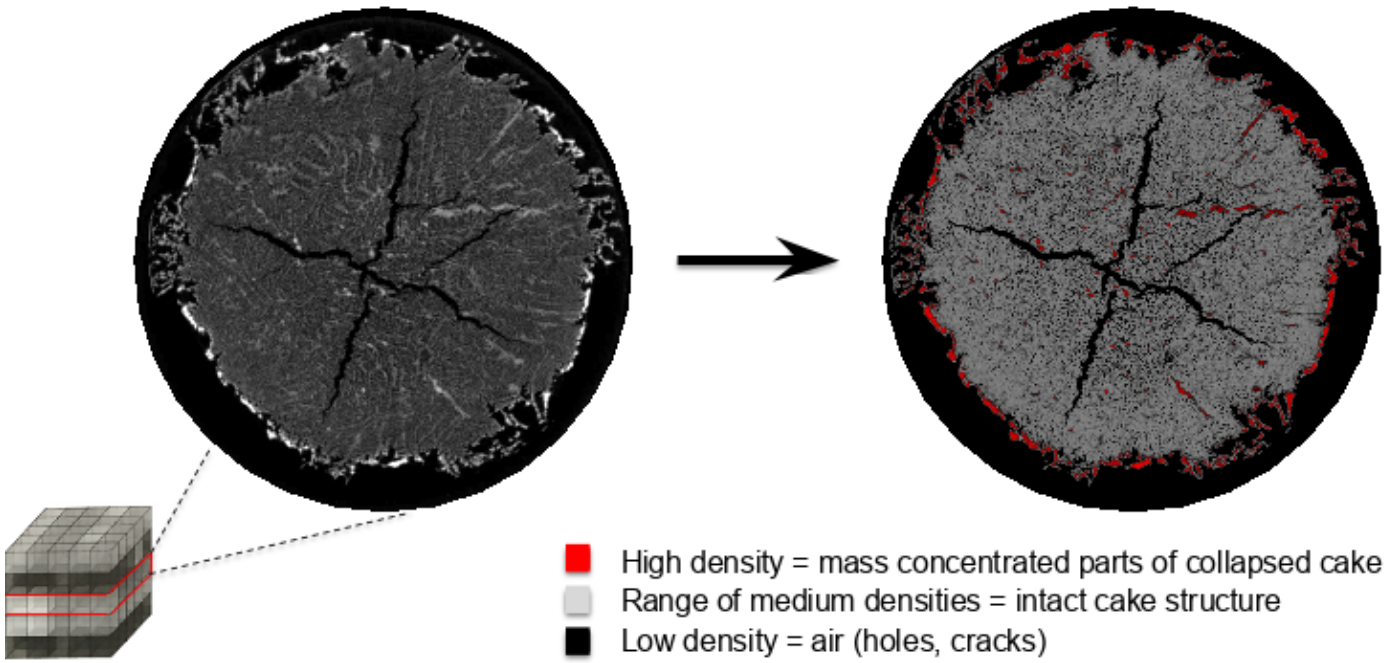
- A micro-focus x-ray source illuminates the object and a planar x-ray detector collects magnified projection images.
- Based on hundreds of angular views acquired while the object rotates, a computer synthesizes a stack of virtual cross section slices through the object.
- You can then scroll through 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)

Global cake characterization

µ-CT - Interpretation of reconstructed volume

Reconstructed *µ*-CT slice **Histogram based coloration**



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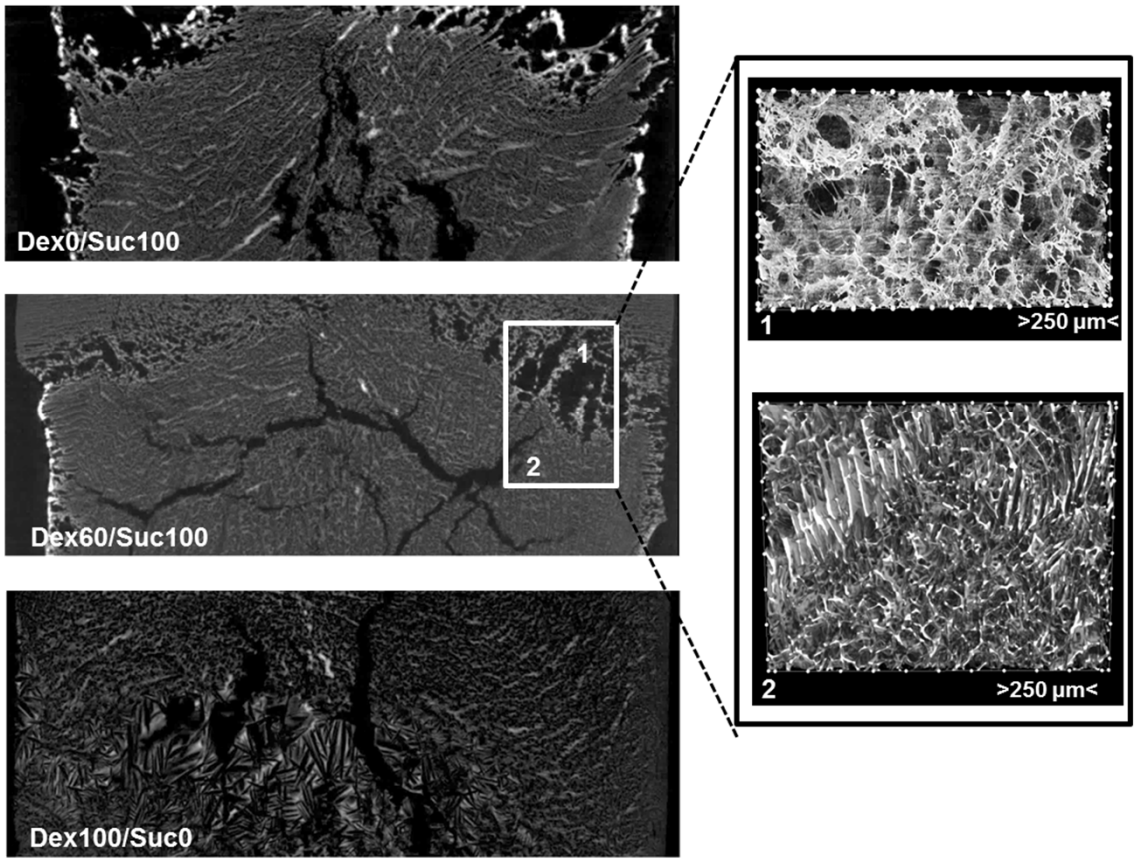
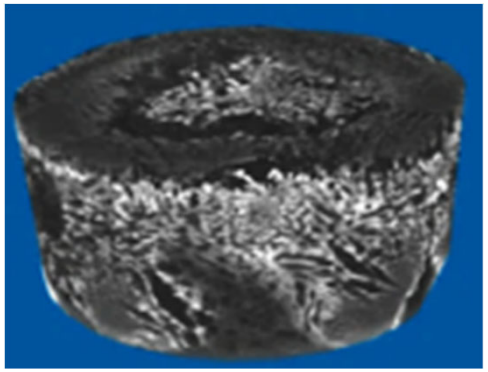


VIDEO



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.