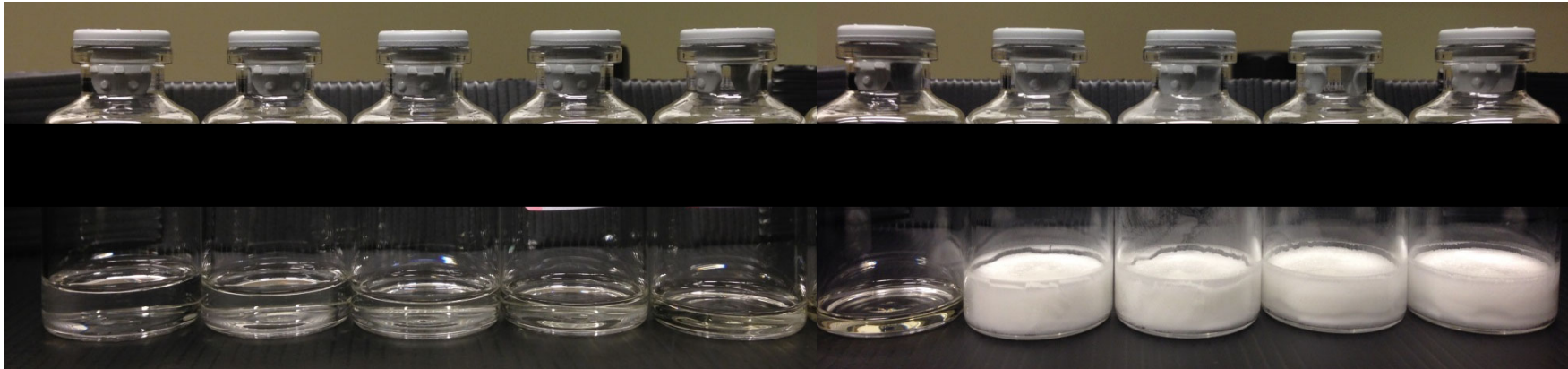


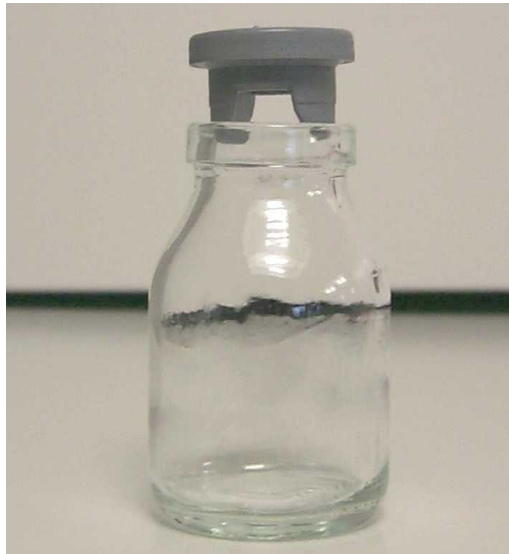


Progress of drying





Primary packaging



Vial
(different coatings)



Cartridge

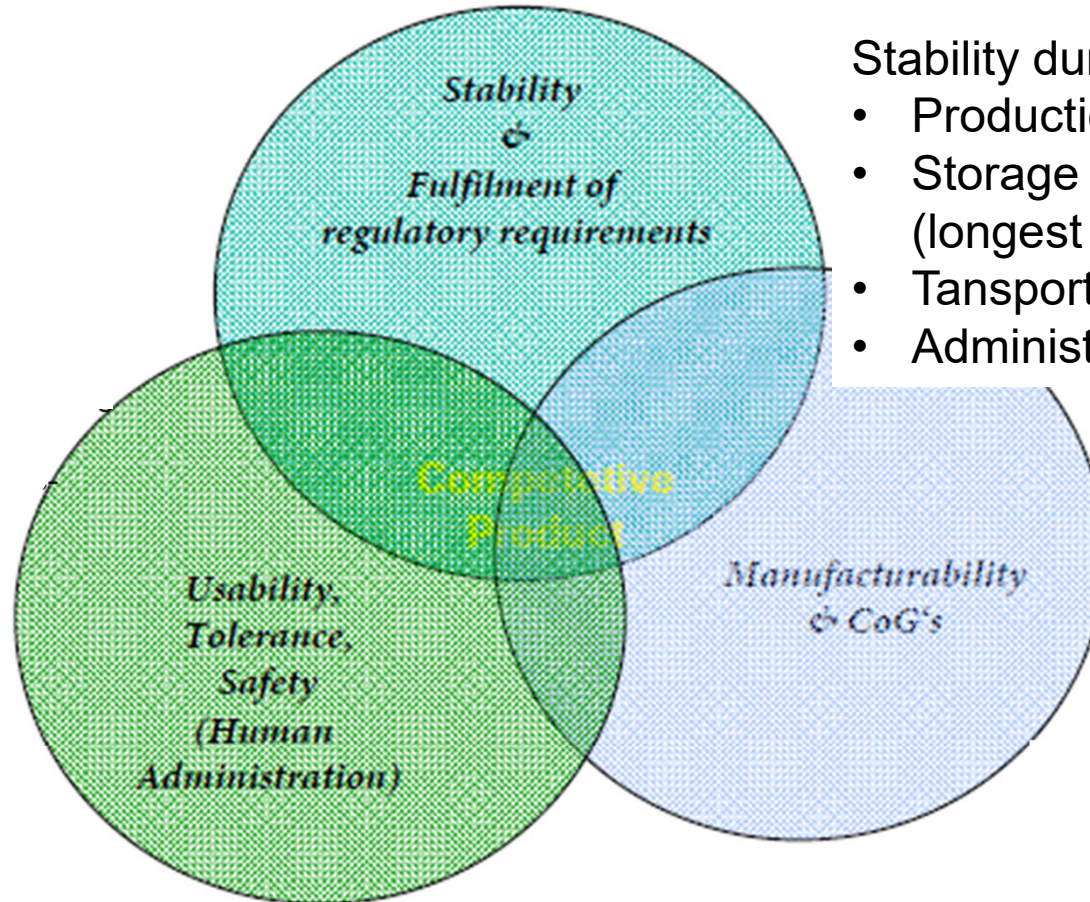


Syringe
(Dual chamber syringe)



Requirements of a formulation

- Patient convenience
- Patient adherence
- Dose delivery



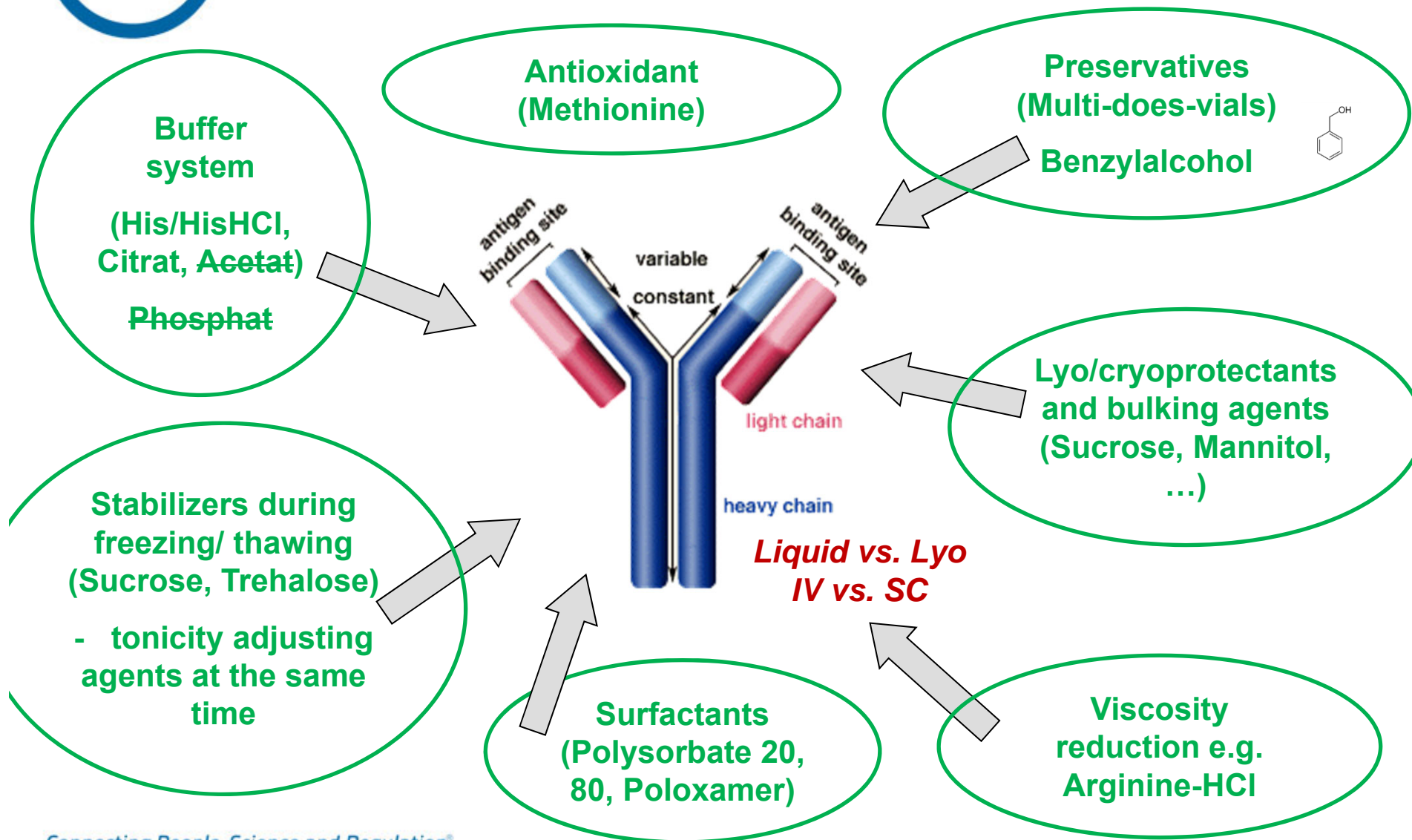
Stability during:

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

Caveat for proteins: Influence on undesirable adverse events and clinical efficiency, immunogenicity and pharmacokinetic profile through product specific degradation products.



Design of a formulation



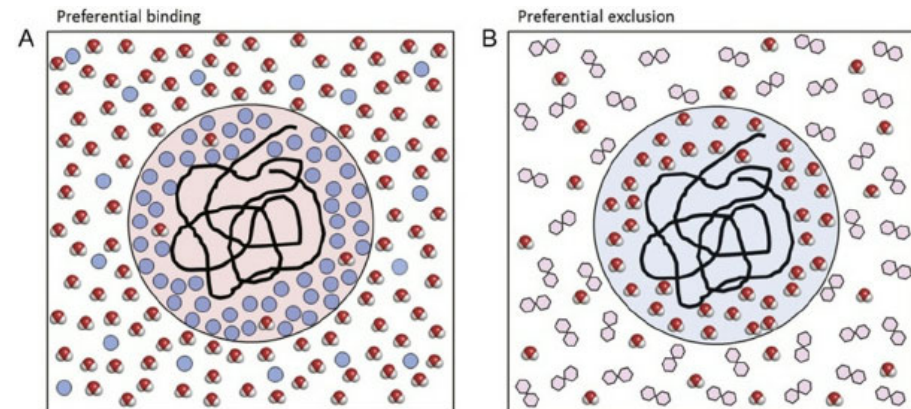


Lyo/cryo-protective excipients

Cryoprotectant

Stabilizes during the freezing process

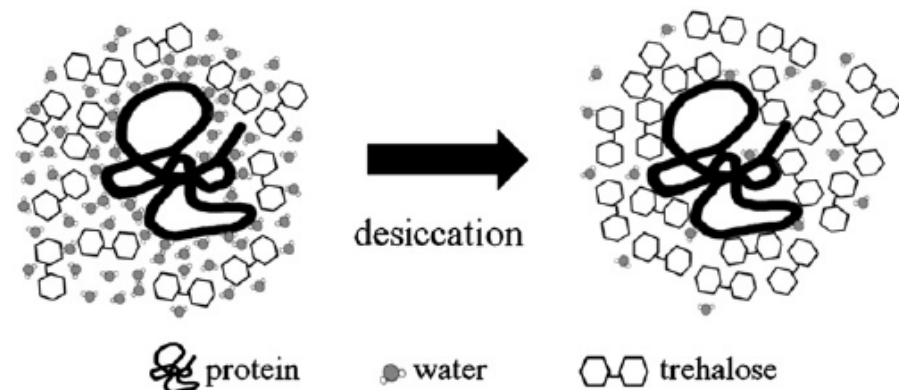
- Excipients are preferentially excluded from the surface of the protein. This is an thermo-dynamically unfavored state. As the unfolded state of the protein would enhance this state, the protein is stabilized.
- (Timasheff 1993).



Lyoprotectant

Stabilizes during the drying process

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

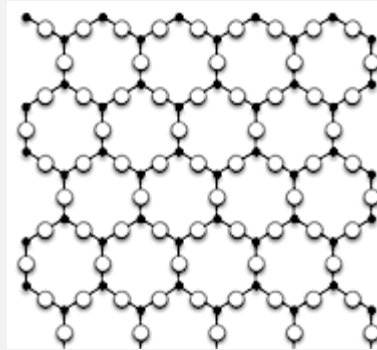




Lyo/cryoprotective excipients

Crystalline excipients

Ordered crystal structure



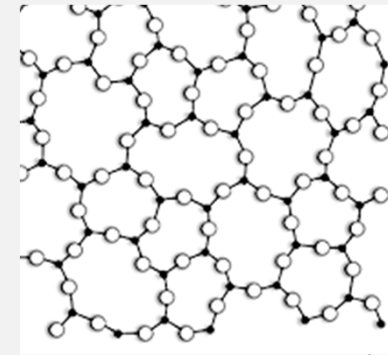
Eutectic temperature
(defined melting point)

- Bulking agent
- High eutectic temperature :
 - Elegant cake appearance
 - Fast drying
- In many cases no stabilization (e.g. for most proteins)
- Different morphologies dependent on excipient (Mannitol → Annealing)
- Glass breakage (Mannitol at high fill)

Glycin, Mannitol, NaCl, ...

Amorphous excipients

Glassy state



Glas transition temperature

Characterization by differential scanning calorimetry

- Stabilization of e.g. proteins
- Acceptable bulking agent at the same time
- Low glass transition temperatures
→ Cake structure?

Sucrose, Trehalose, 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 % Polysorbat 20





Analytical characterization

Product attributes for designing lyophilization cycles

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

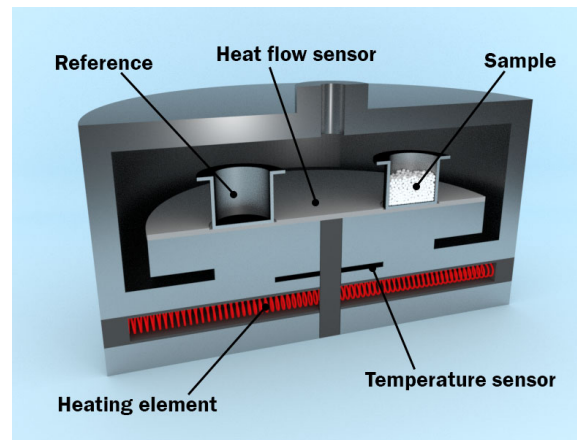
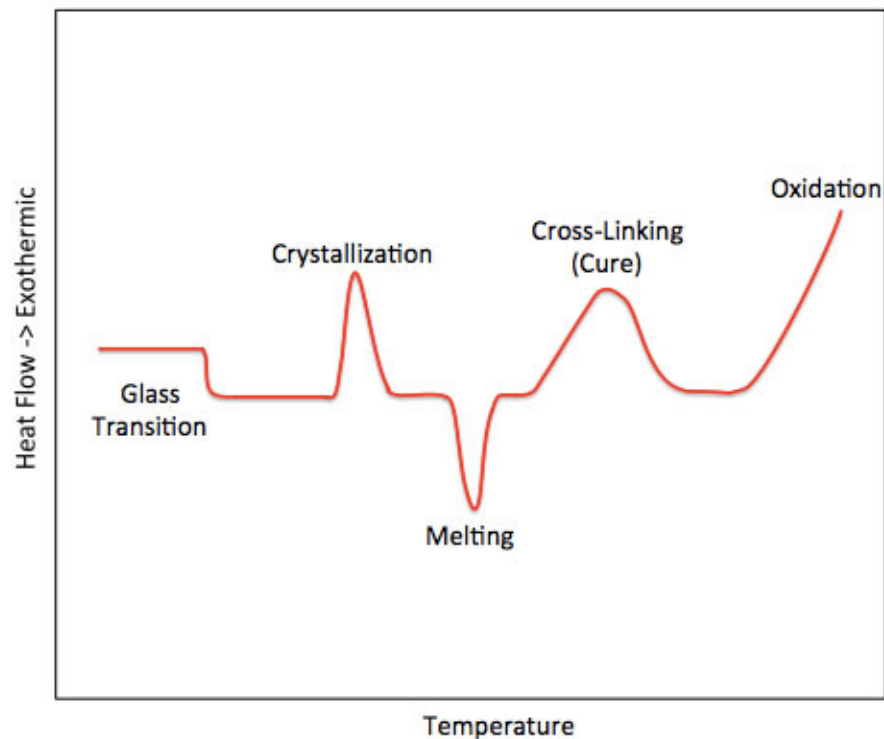
Solid state characterization after lyophilization

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

Other quality attributes of active compound



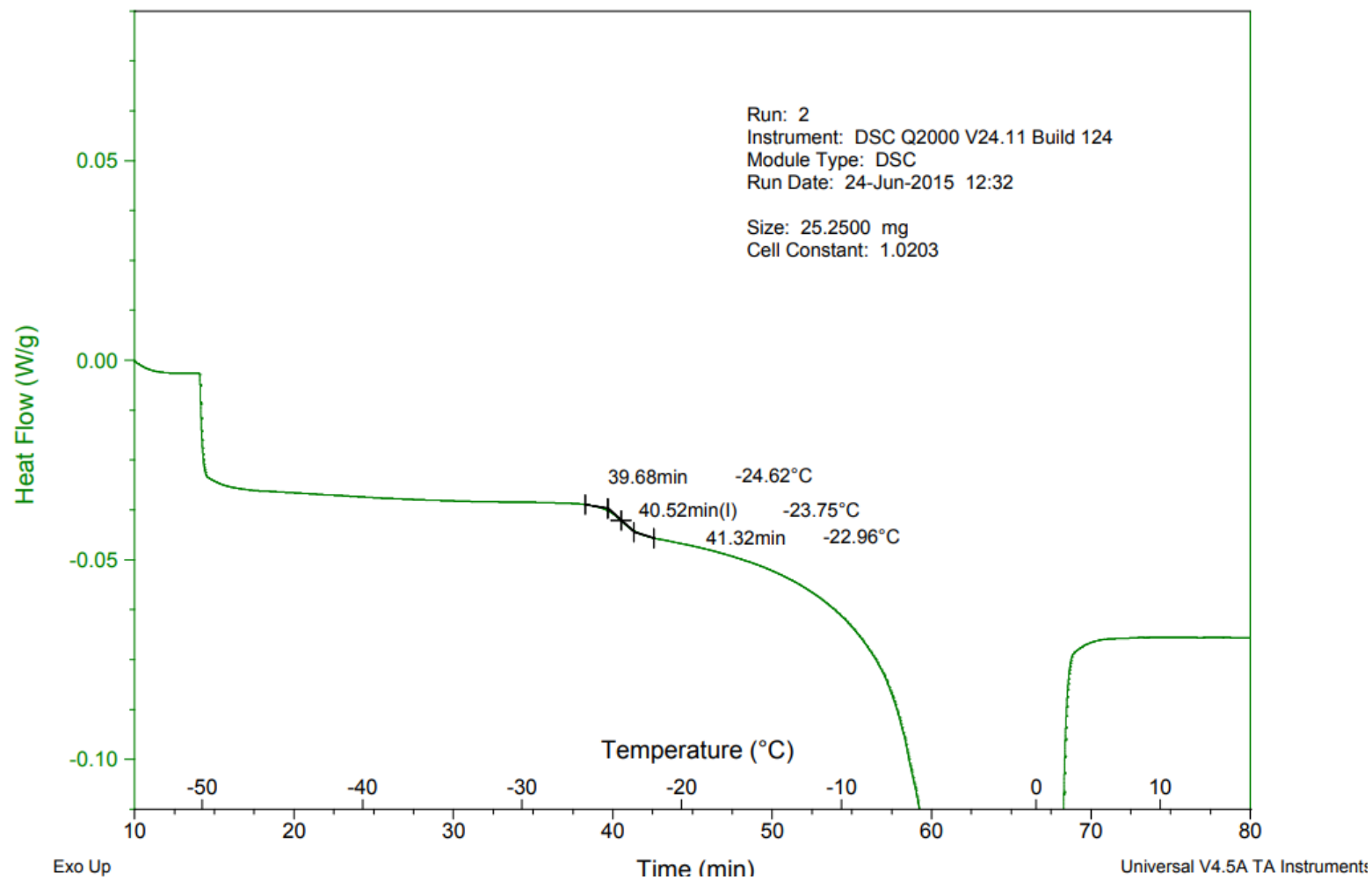
Differential Scanning Calorimetry (e.g. T_g)



- 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

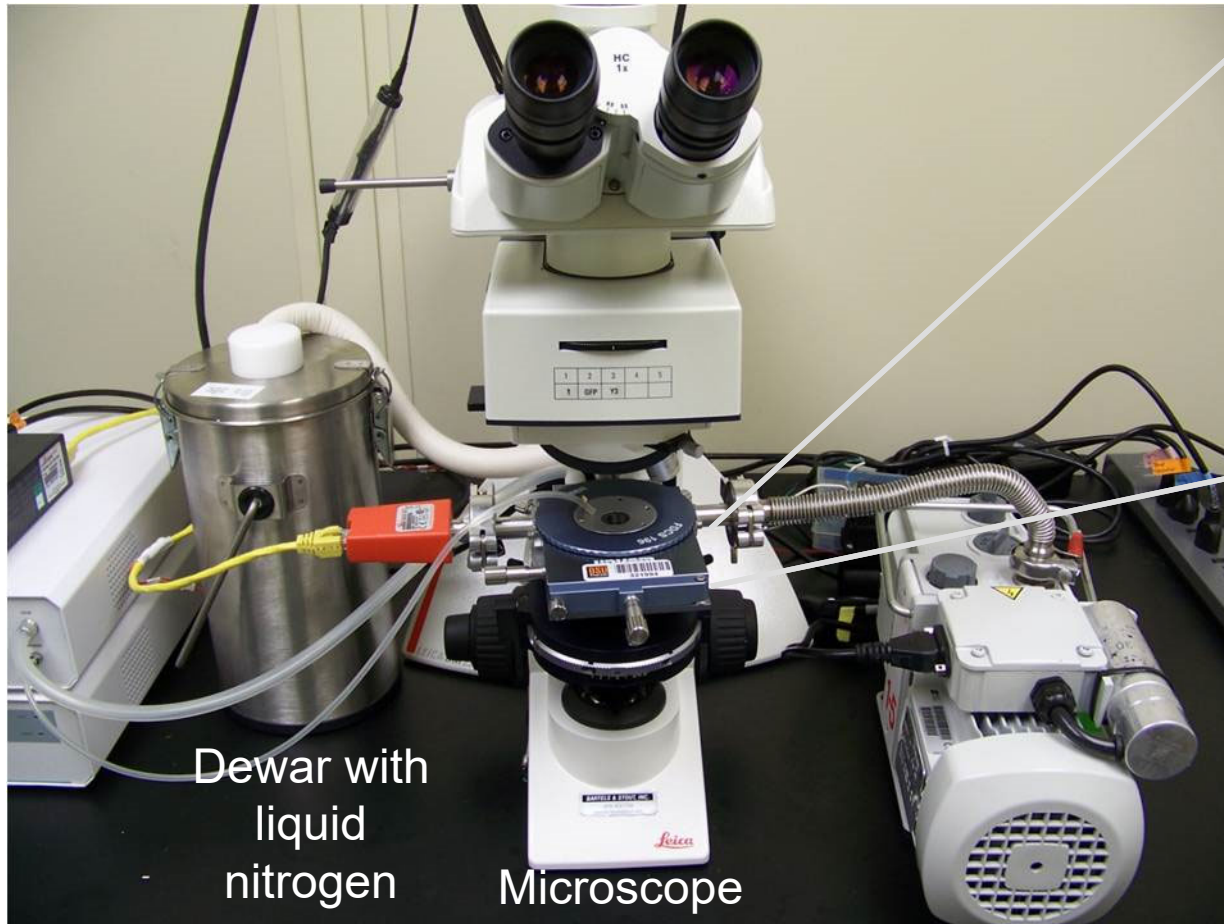


Differential Scanning Calorimetry (e.g. T_g)





Freeze drying microscopy ($T_{collapse}$)



Cryostage

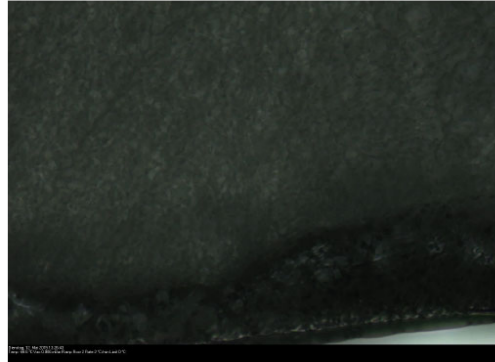
Vacuum pump



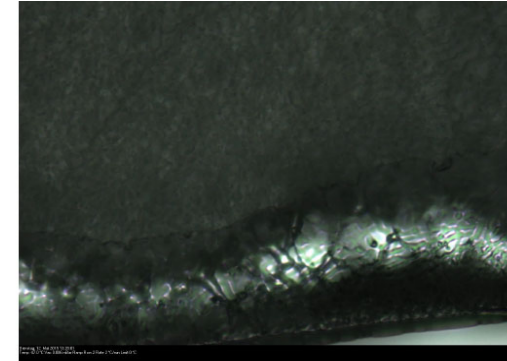
Freeze drying microscopy (T_{collapse})



(Intact) frozen sample



Onset of collapse



Complete collapse

$\rightarrow T_g < T_{\text{collapse}} !!$



Residual moisture – Water content



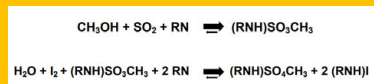
Gravimetric analysis

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

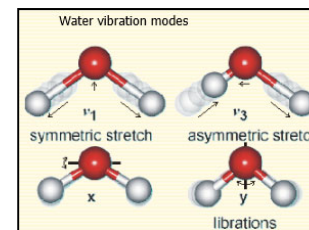


Karl-Fischer

- Quantitative water determination by titration



- 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 I₂/I⁻ redox couple).

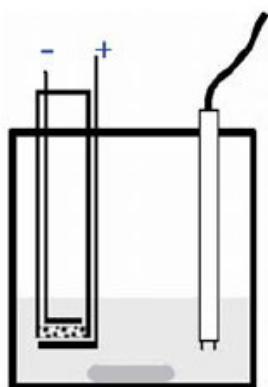


Volumetric Karl Fischer Titration

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



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%**

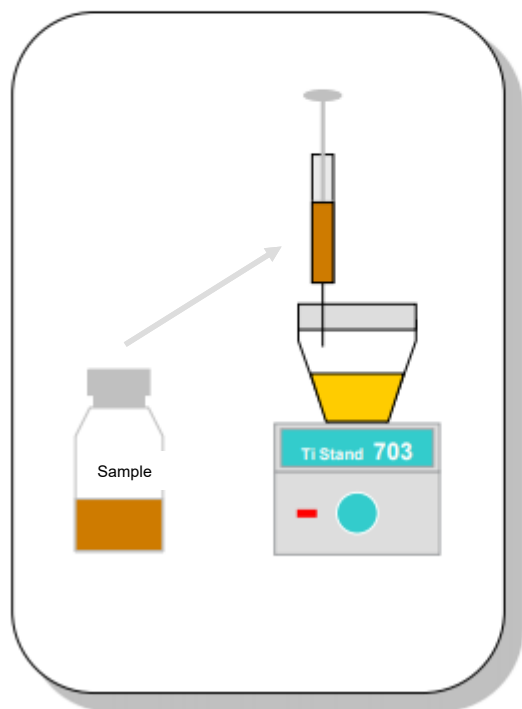
- The working medium consists of the components sulfur dioxide, alcohol, and organic base or/and water free vehicle.
- Two electrodes are needed: One for iodine generation (anode), and one for potentiometric end-point detection via the indicator electrode (free I₂/I⁻ redox couple).



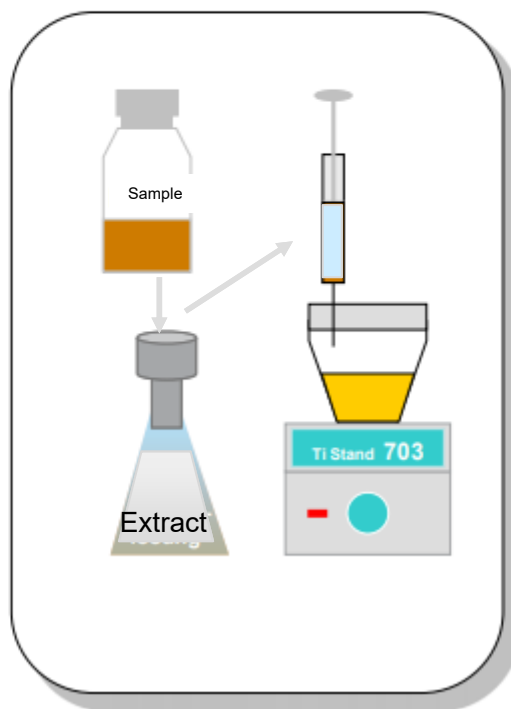


Karl-Fischer Titration

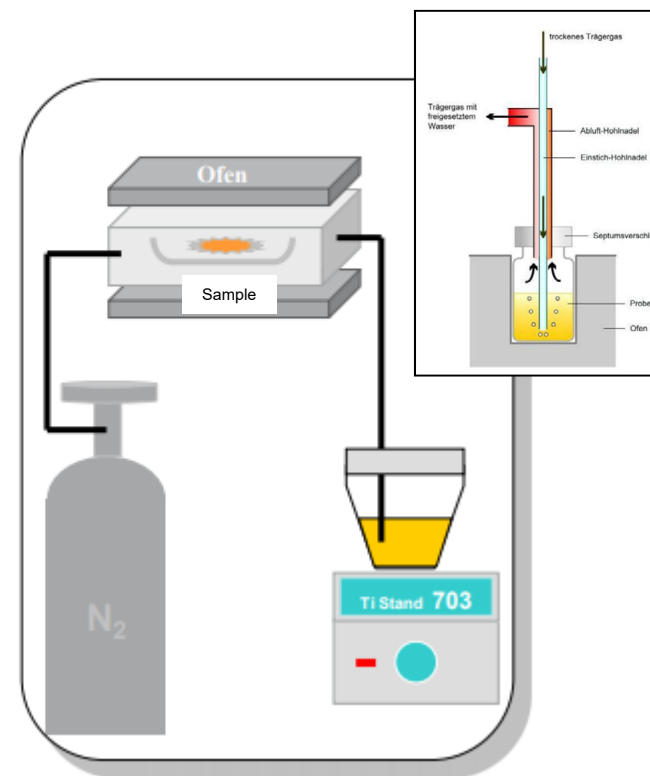
Direct Titration



Liquid Extraction



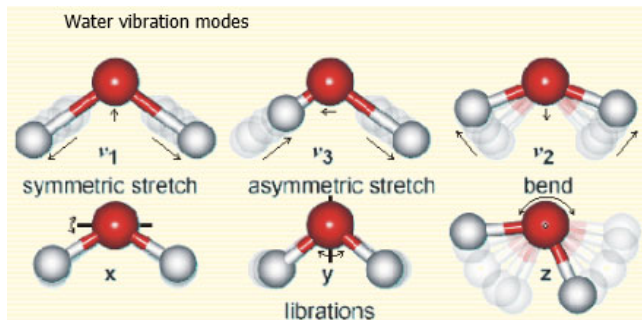
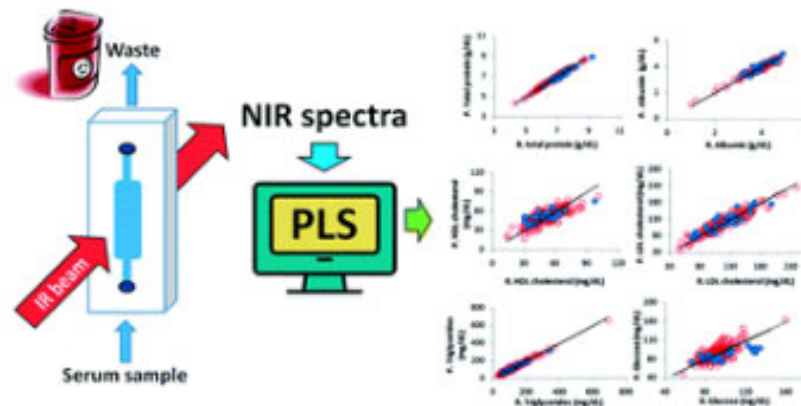
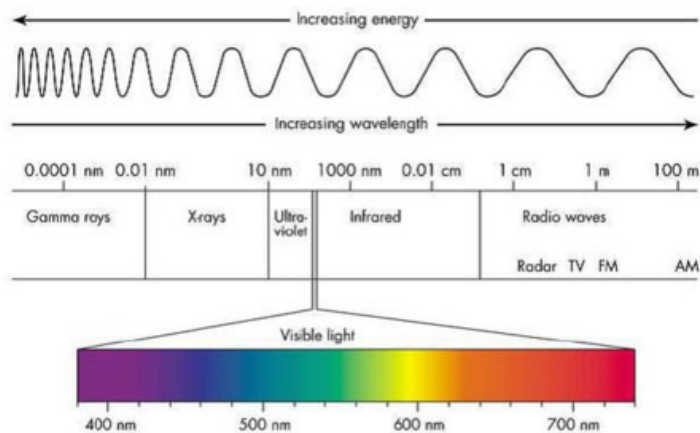
Evaporation



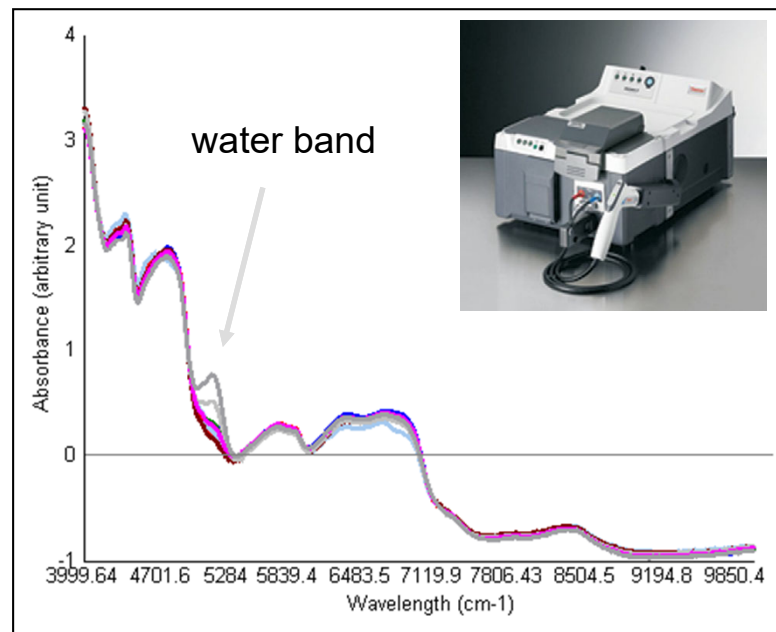
Highly dependent on the sample and its heat sensitivity.



Residual moisture - NIR



- Molecule vibrations (overtone and combinations)
- Near infrared: $\sim 760\text{--}2500\text{ nm}$ or $13.000\text{--}4.000\text{ cm}^{-1}$





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)
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(visual inspection, 3D scanning, PDMS embedding, SEM, μ CT)

Other quality attributes of active compound



Reconstitution time

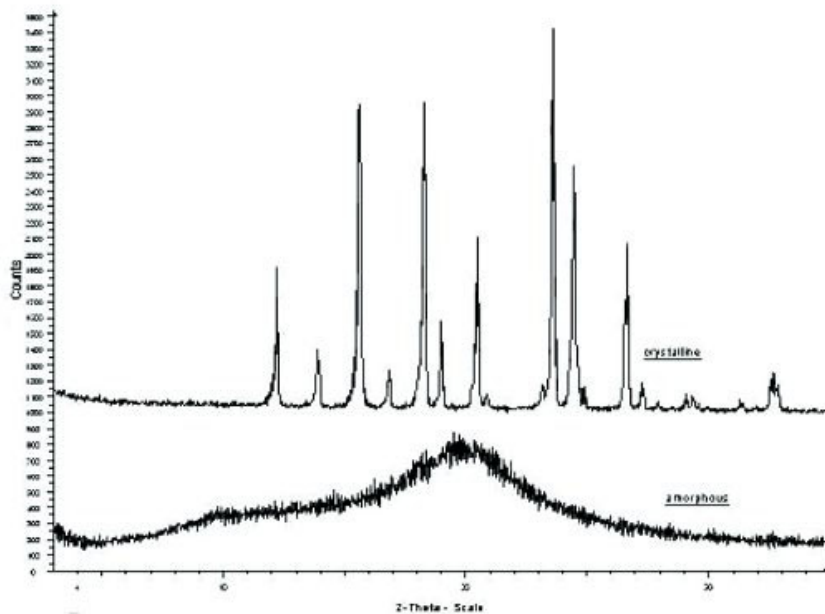


- Water ideally flows along the side wall
- Avoid foaming if samples contain surfactants
- In case of long reconstitution times, shaking systems may be considered

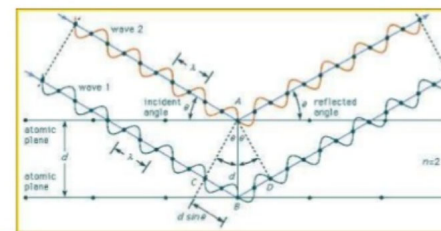
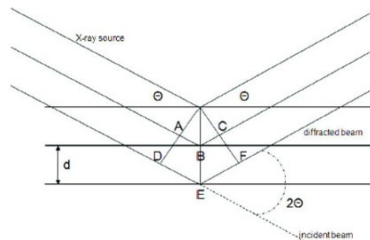


Xray powder diffraction - Morphology

- 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



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



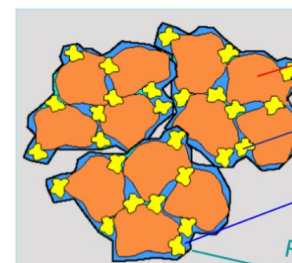
Bragg's law:
 $n\lambda = 2d\sin\theta$

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

Mixture analysis

Patterns are additive

Multi-phase sample

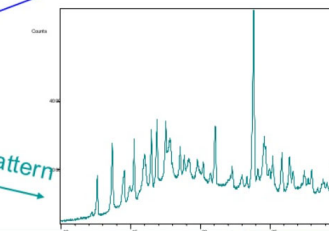
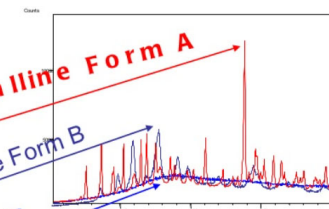


Crystalline Form A

Crystalline Form B

Amorphous

Resulting XRD pattern



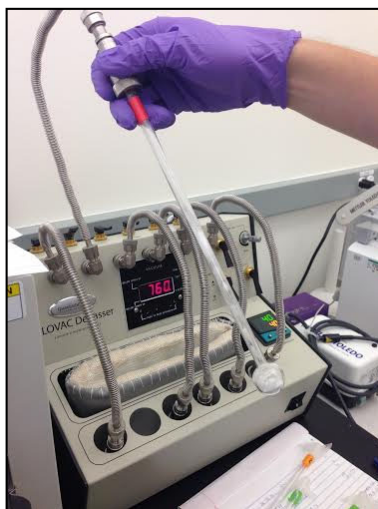


Specific surface area (BET)

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: degassing under vacuum and elevated temperature followed by measurement in liquid N₂.



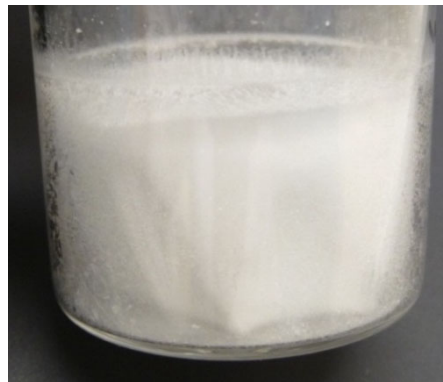
Visual inspection

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



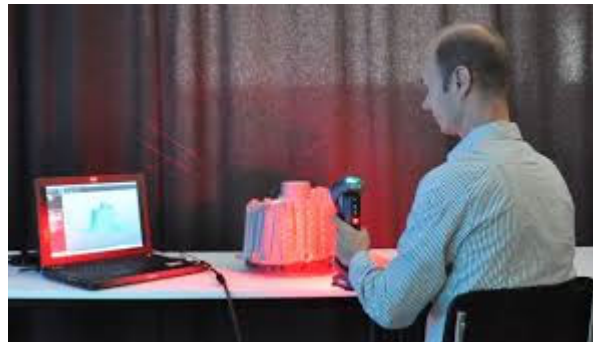
splashing



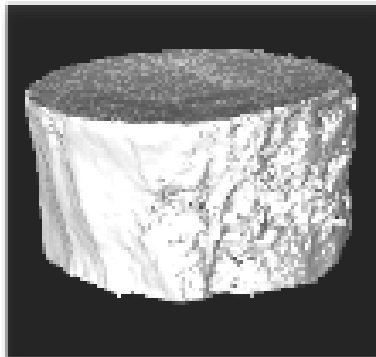
fogging



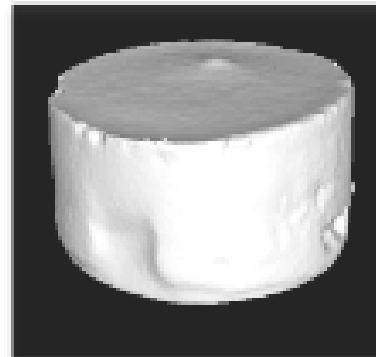
3D scanning



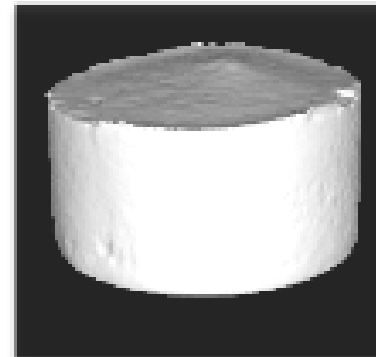
Dex0/Suc100



Dex60/Suc40



Dex100/Suc0





PDMS embedding

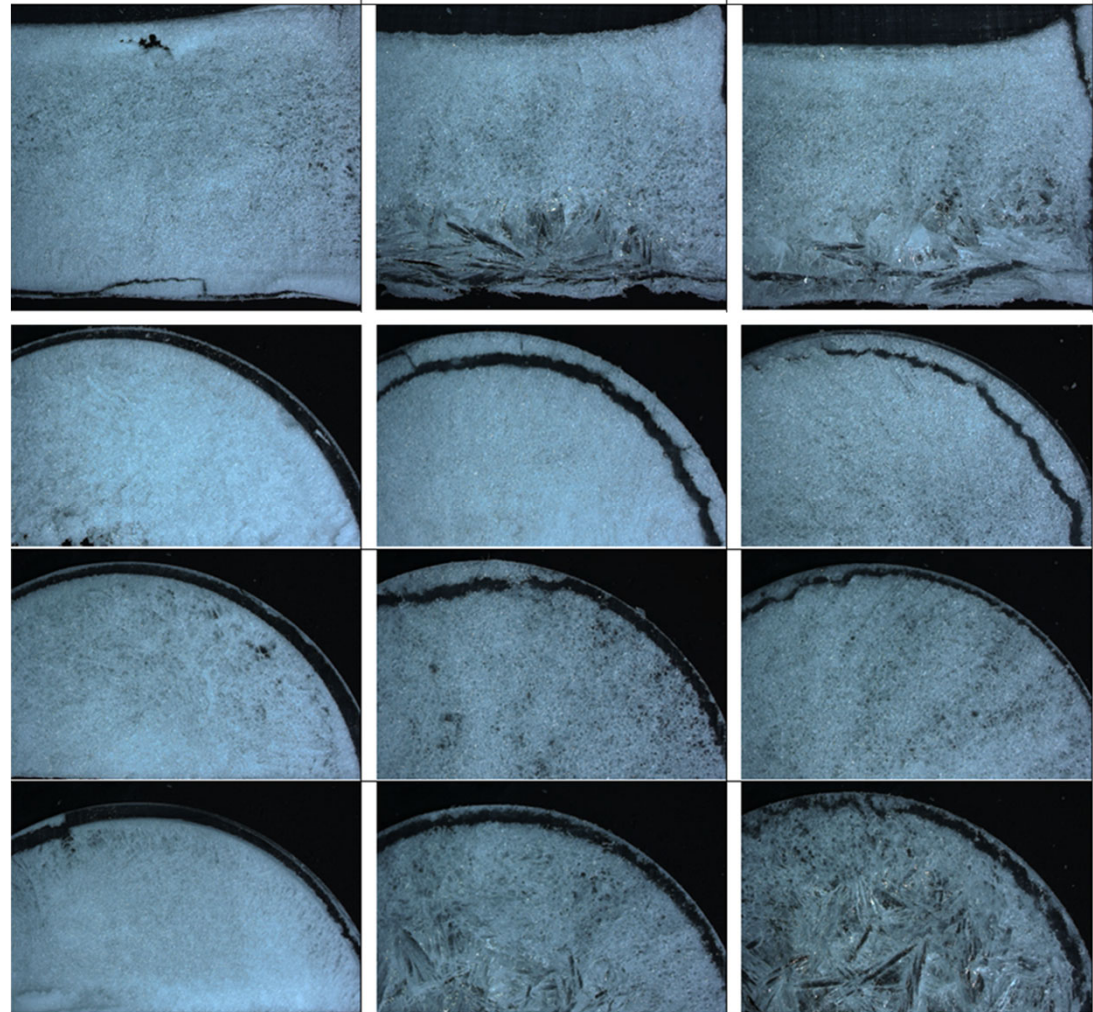
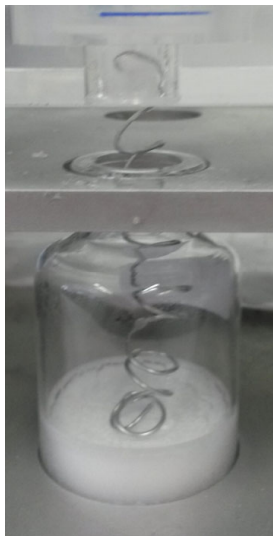
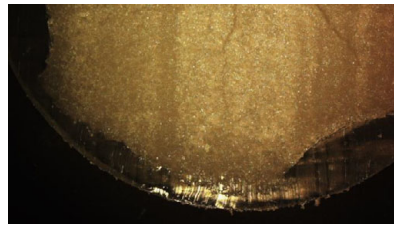
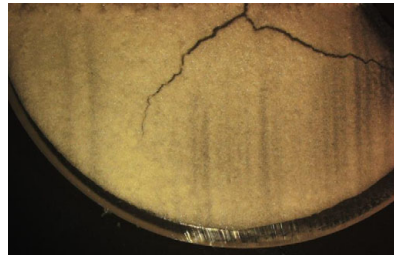
PDA Journal
of Pharmaceutical Science and Technology



An Improved Method for Visualizing the Morphology of Lyophilized Product Cakes

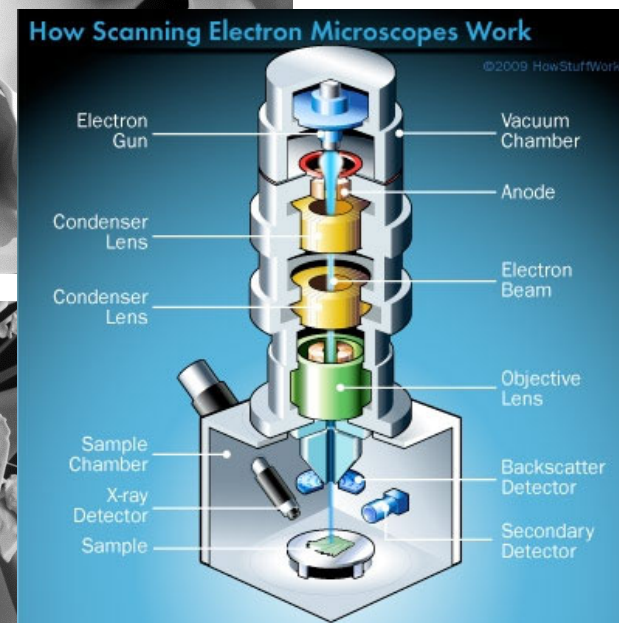
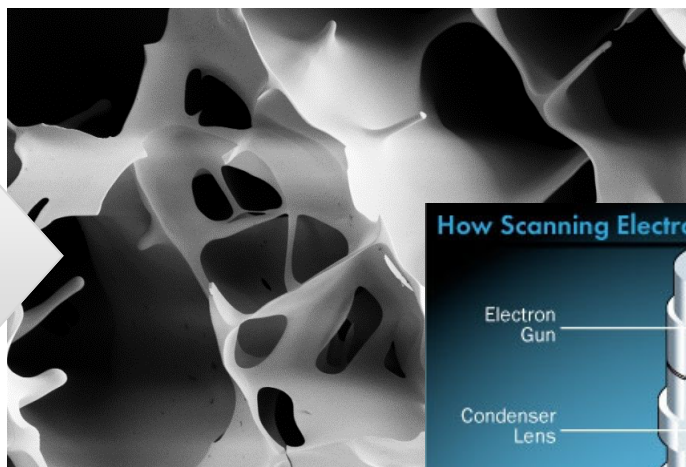
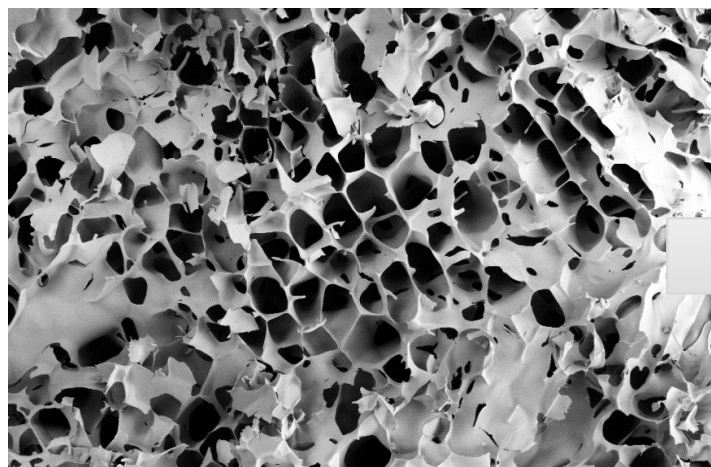
Philippe Lam and Thomas W. Patapoff

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





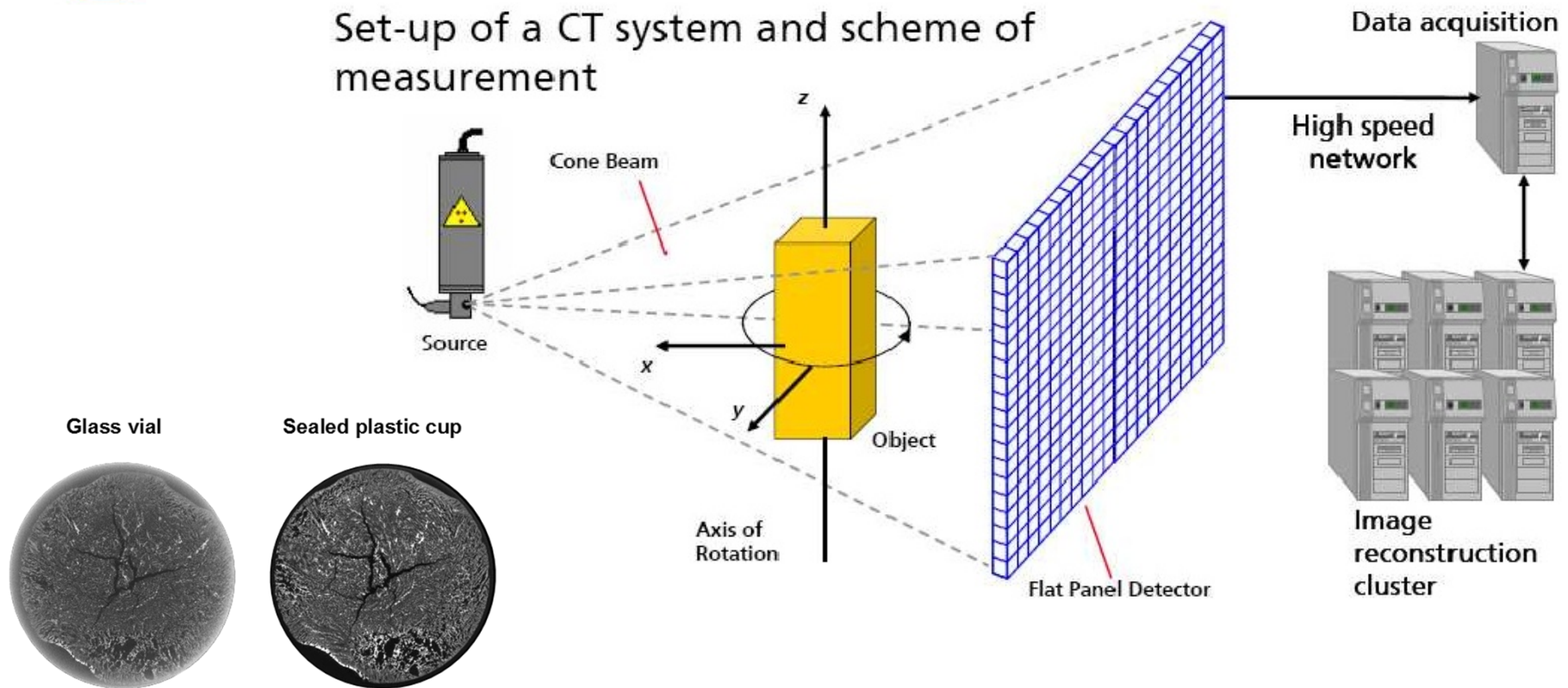
Scanning electron microscopy (SEM)





Micro-computed tomography (μ CT)

Set-up of a CT system and scheme of measurement



- 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.



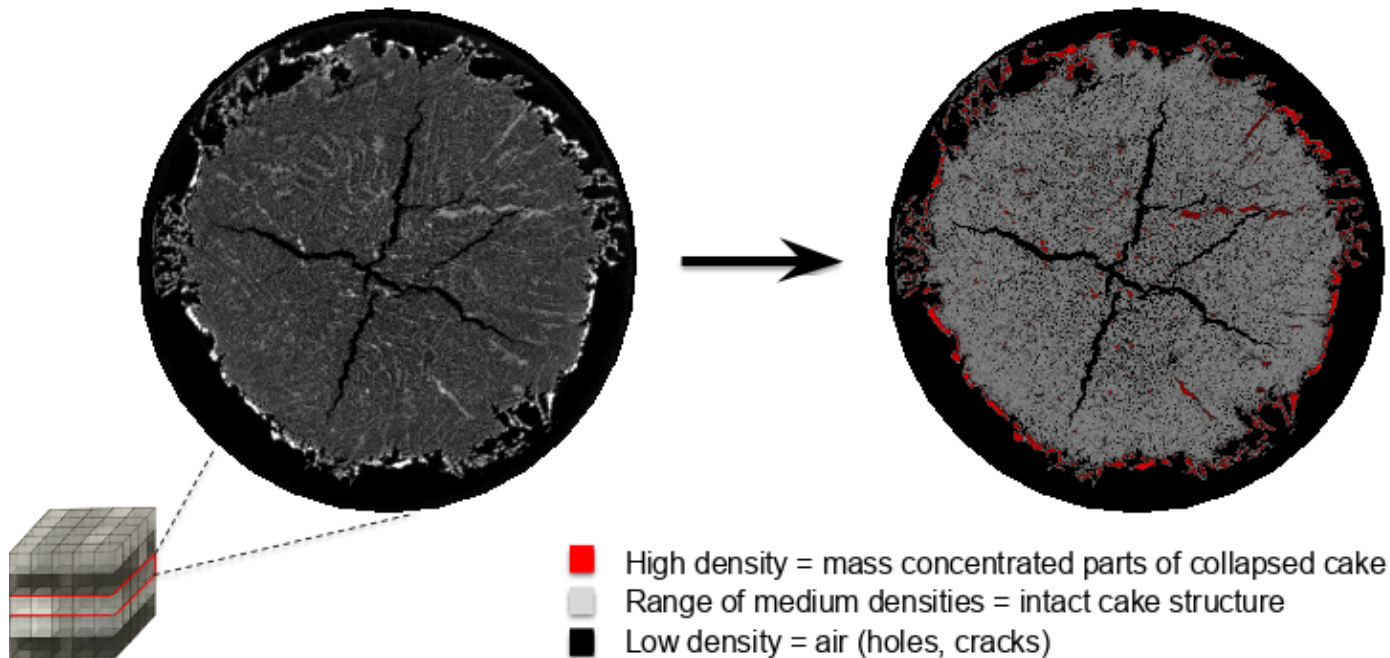
Micro-computed tomography (μ CT)

Global cake characterization

μ -CT - Interpretation of reconstructed volume

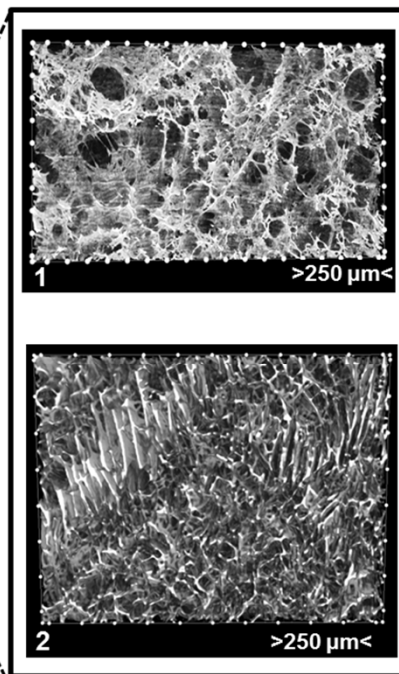
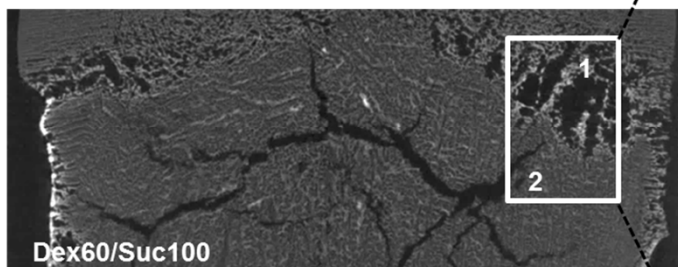
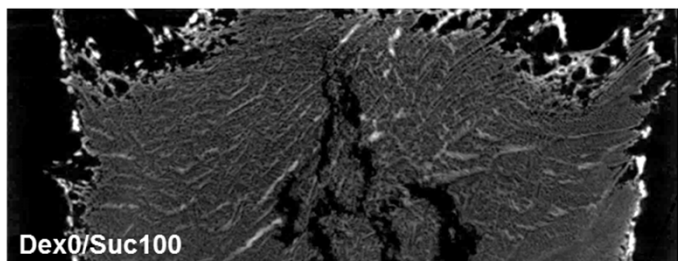
Reconstructed μ -CT slice

Histogram based coloration





Micro-computed tomography (μ CT)



VIDEO

