Scanning Electron Cryomicroscopy (Cryo-SEM) For Characterization of Complex Drug Products

SBIA 2021: Advancing Generic Drug Development: Translating Science to Approval

Day 2, Session 1: (Cutting Edge Science)

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CDER | U.S. FDA

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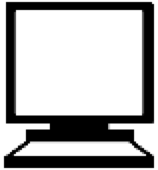
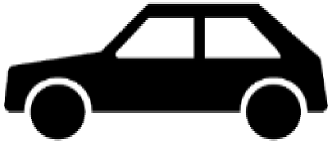
Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.





Pharmaceutical Quality

A quality product of any kind consistently meets the expectations of the user.

Drugs are no different.

Patients expect safe and effective medicine with every dose they take.

Pharmaceutical quality is

assuring *every* dose is safe and effective, free of contamination and defects.

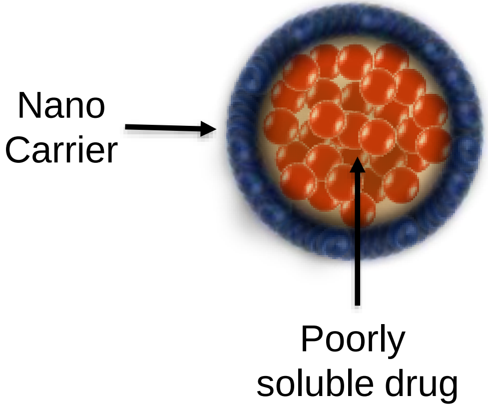
It is what gives patients confidence in their *next* dose of medicine.

Learning Objectives

- Describe what is considered a nanomaterial and why is electron microscopy used or needed
- List four techniques used to make particle size measurements in nanomaterial containing complex drugs
- Distinguish image-based quantitative analysis and particle size analysis based on light scattering
- Evaluate sample preparation approaches and particle sizing techniques for size and shape analysis of nanomaterials

Nanotechnology in Medicine

- "Application of nanotechnology"
  - Nanoscale range: 1-100 nm
  - Size-related phenomena: ≤ 1000 nm
- Increase potency
- Decrease toxicity
- Improve delivery



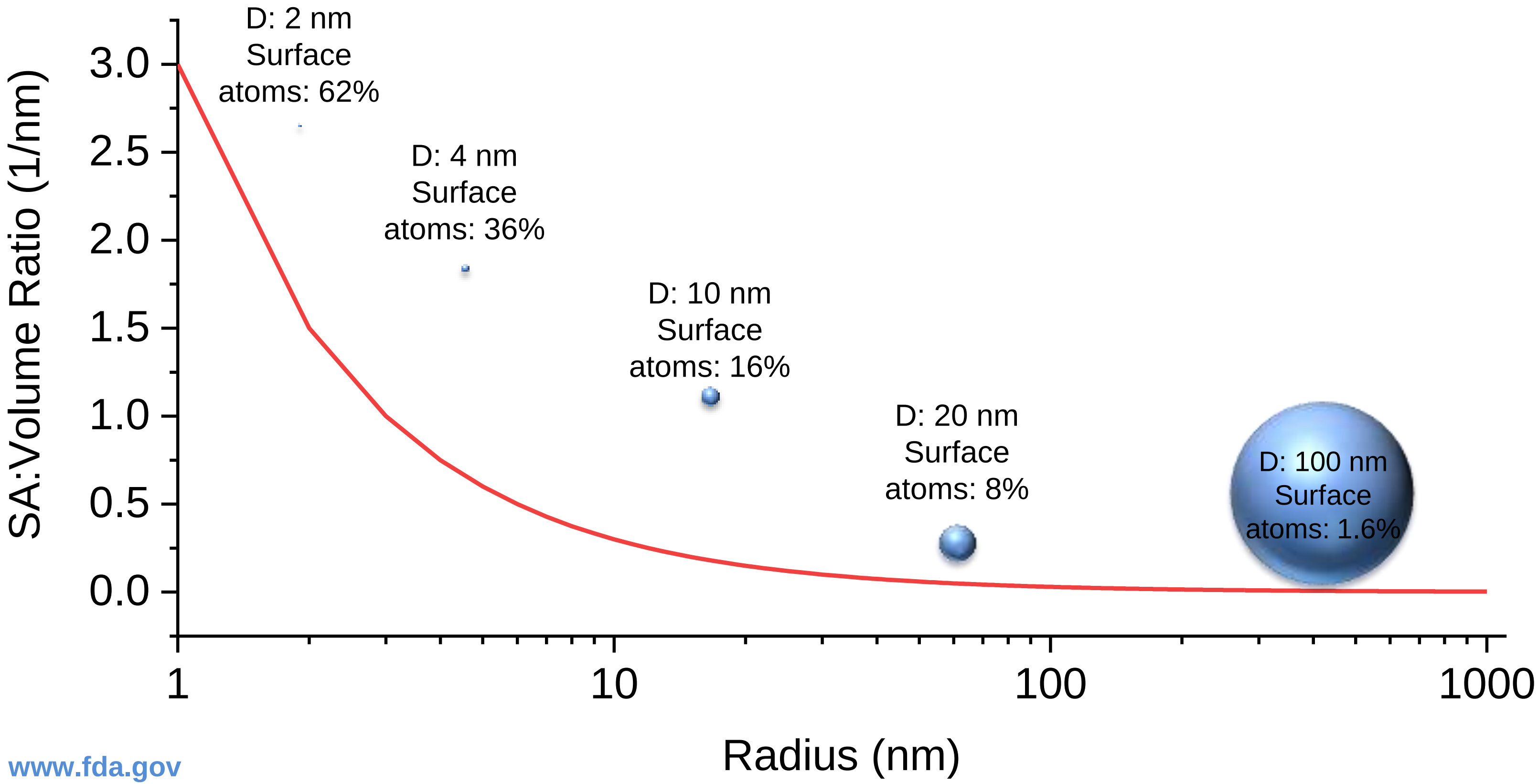
Size

Shape

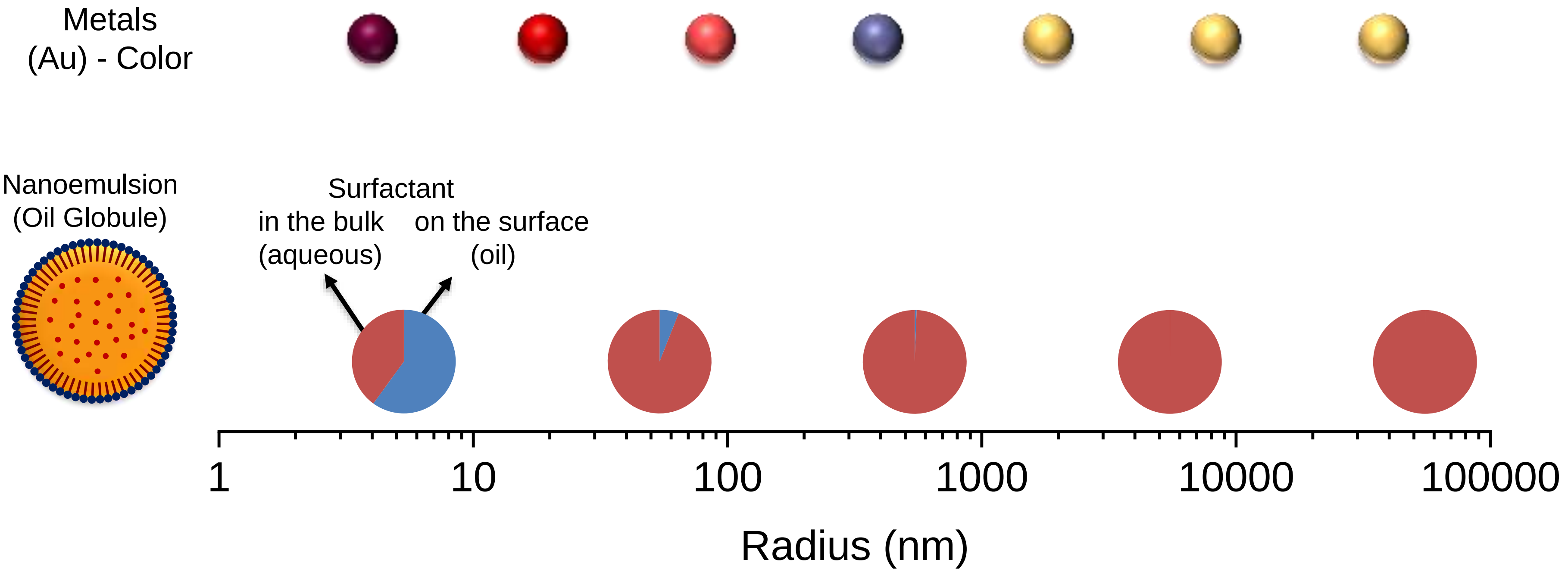
Surface Chemistry

Composition

Nanoparticles



Nanoparticles – Properties vs Size

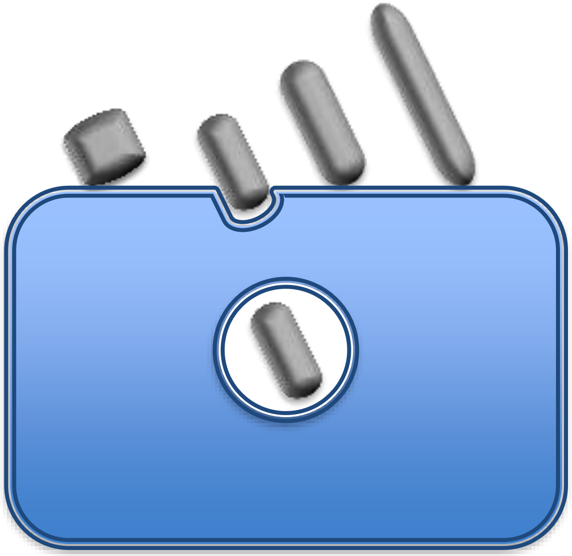


Nanoparticles – Properties vs Shape

Aspect Ratio

Metals (Au) - Color

Nanocrystalline API – Cellular Uptake



Aspect Ratio Determines the Quantity of Mesoporous Silica Nanoparticle Uptake by a Small GTPase-Dependent Macropinocytosis Mechanism

VOL. 5 ▪ NO. 6 ▪ 4434–4447 ▪ 2011 ACSNANO
www.acsnano.org

From the journal
Nanoscale

The aspect ratio effect of drug nanocrystals on cellular internalization efficiency, uptake mechanisms, and *in vitro* and *in vivo* anticancer efficiencies†

Analytical Considerations: Light Microscopy

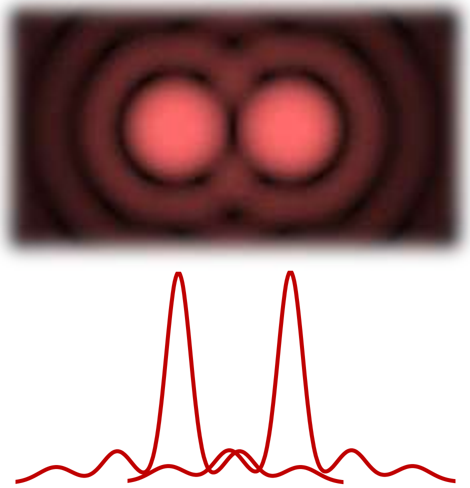
- Wave nature of light, resolution:

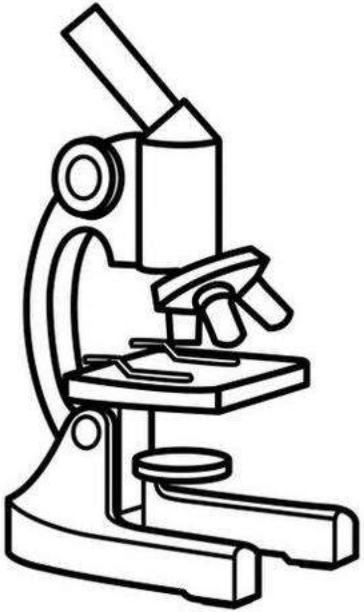
$$\frac{\lambda}{2n\sin\theta} = \frac{\lambda}{2NA}$$

- $NA \leq 1.51$ (realistically ≤ 1.4)

- For dry objectives $NA \leq 1$

- At 550 nm (green light) resolution $\geq 200\ nm$ (realistically 1-5 µm)



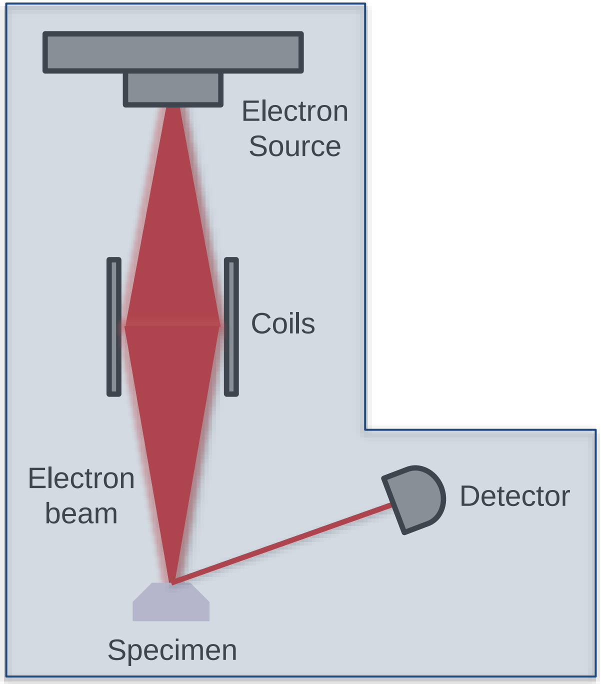


Analytical Considerations: Electron Microscopy

- Electron microscopy:

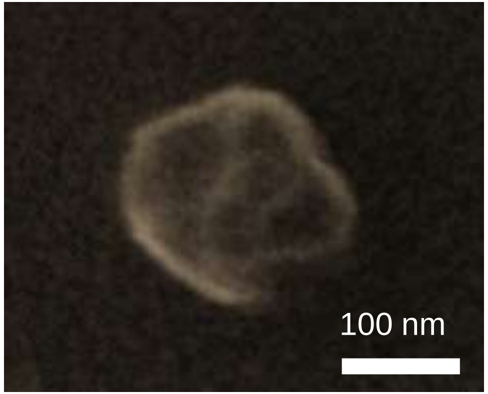
$$\lambda = \frac{h}{\sqrt{2meV}}$$

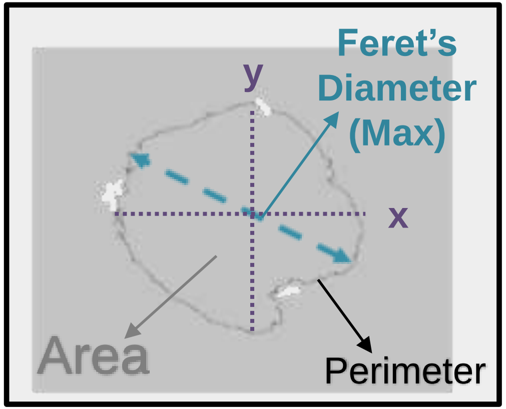
- $\sim 4\ pm$ at 100 keV, $\sim 12\ pm$ at 10 keV
- TEM, SEM, STEM, STM
- SEM: 0.4 nm (realistically 1-10 nm)



Vacuum

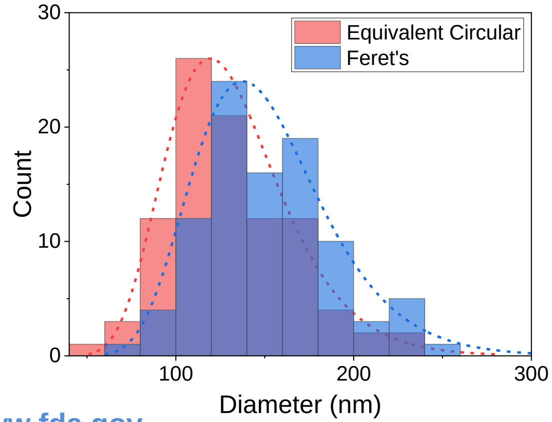
Image Based Quantitative Analysis

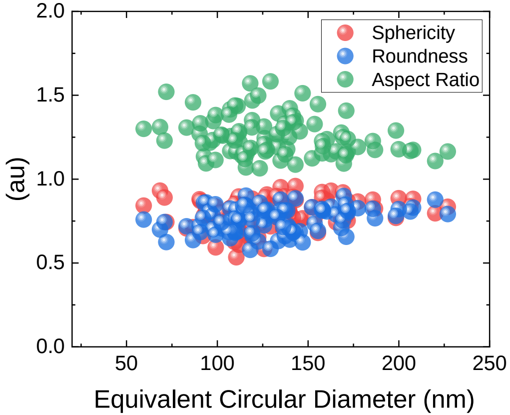




$$D = 2\sqrt{\frac{Area}{\pi}}$$

$$AR = \frac{(Feret\ Length)^2}{Area}$$



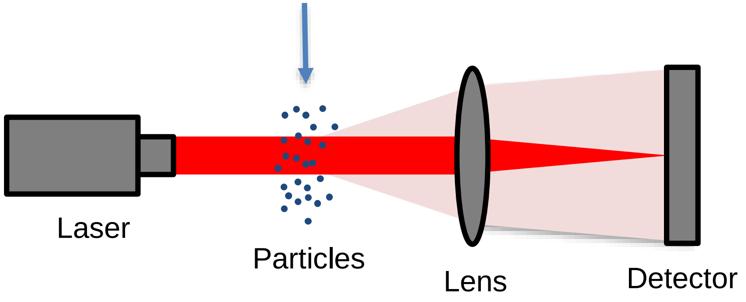


$$Roundness = \frac{4 \times Area}{\pi \times Length^2}$$

$$Sphericity = 4 \times \pi \times \frac{Area}{Perimeter^2}$$

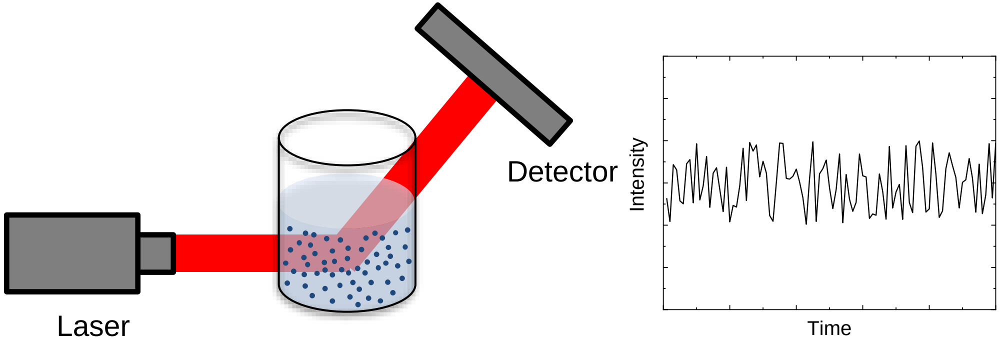
Nanomedicines: Measuring Particle Size

- Static Light Scattering (Laser Diffraction):
  - Measure scattered light intensity and angle
  - Estimate size using Fraunhofer or Mie theory

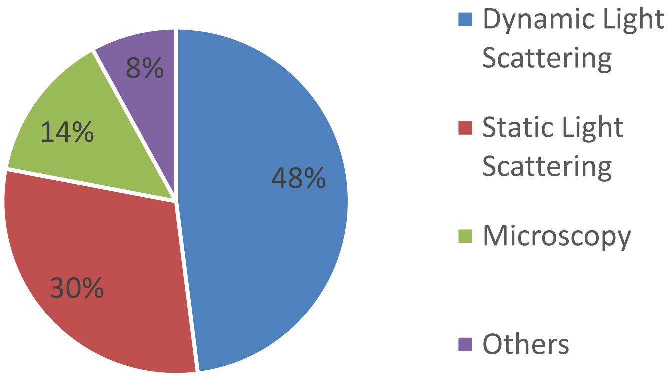


- Dynamic Light Scattering:
  - Brownian Motion
  - Stokes-Einstein Equation:

$$D = \frac{k_B T}{6\pi\eta R_h}$$



Nanomedicines: Particle Size



nature nanotechnology
ANALYSIS
PUBLISHED ONLINE: 24 APRIL 2017 | DOI: 10.1038/NNANO.2017.67

The evolving landscape of drug products containing nanomaterials in the United States

Sheetal R. D'Mello, Celia N. Cruz, Mei-Ling Chen, Mamta Kapoor, Sau L. Lee and Katherine M. Tyner*

- Light scattering methods
  - Quick and easy indirect measurement
  - Limitations with particle shape, sample preparation and model parameters
- Imaging
  - Tedious sample preparation, analysis volume
  - Image based measurement and morphology

Challenge Question #1

Which technique is best suited for characterization of a high aspect ratio (AR > 2) nanocrystal formulation with particles smaller than 50 nm in size (D90 < 50 nm)?

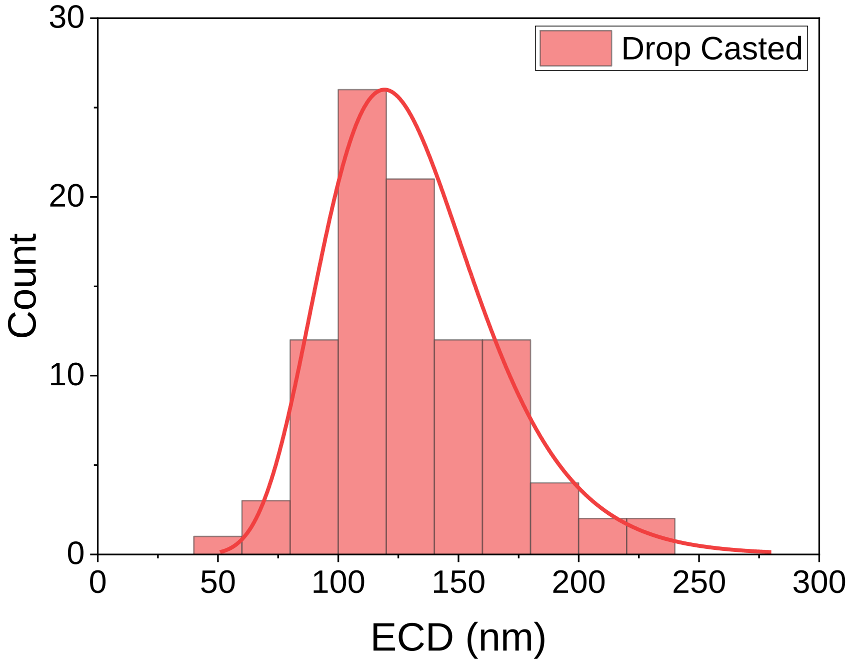
A. Optical Microscopy

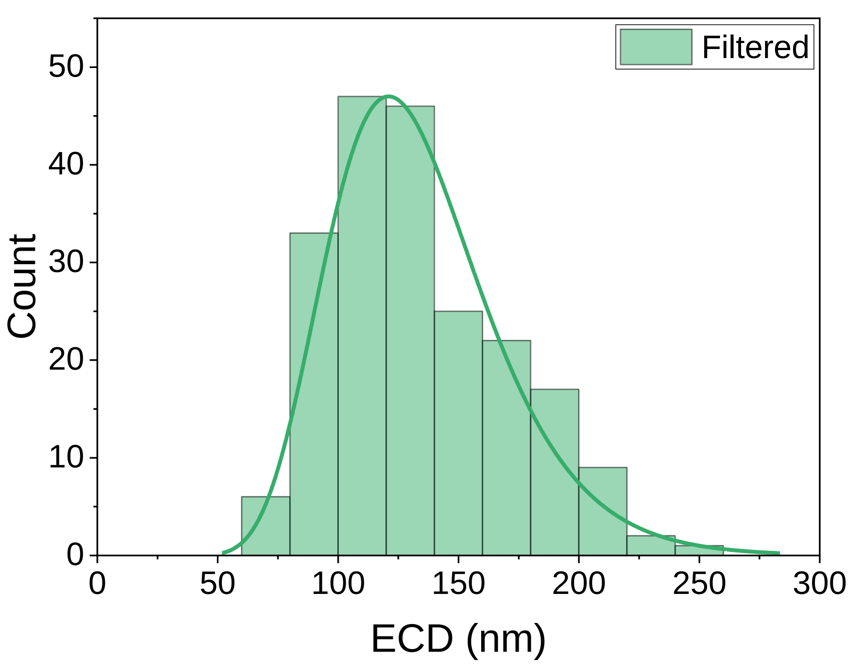
B. Static Light Scattering

C. Dynamic Light Scattering

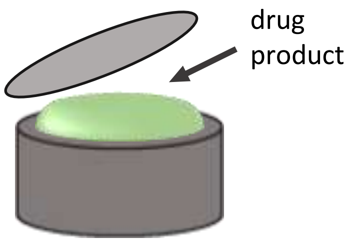
D. Transmission Electron Microscopy

Sample Preparation in EM

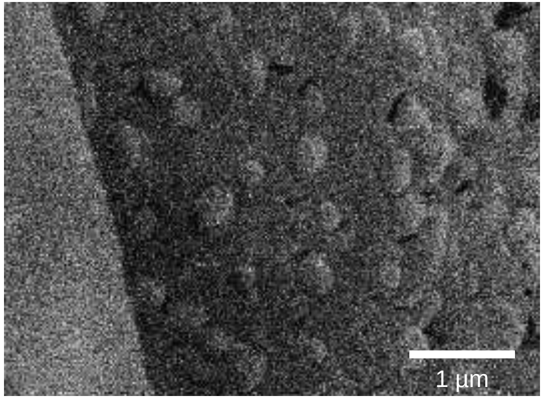


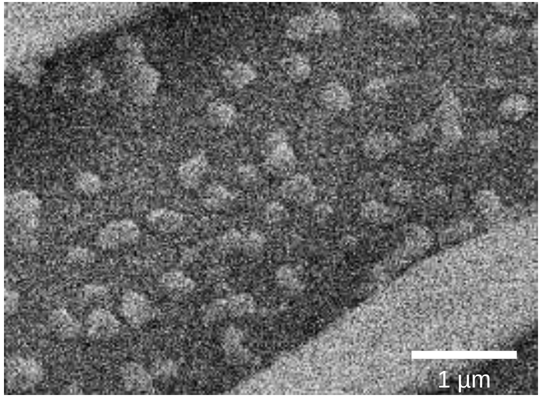


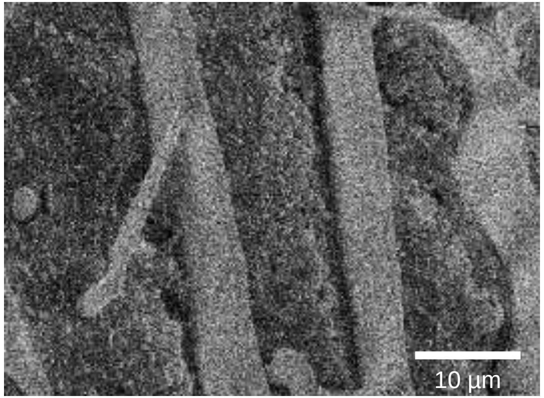
Plunge Freezing

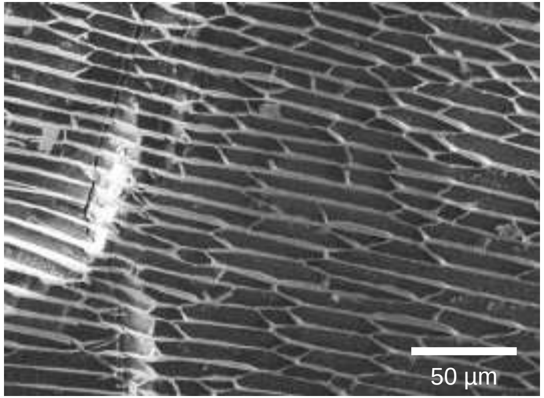


Nitrogen
Slush

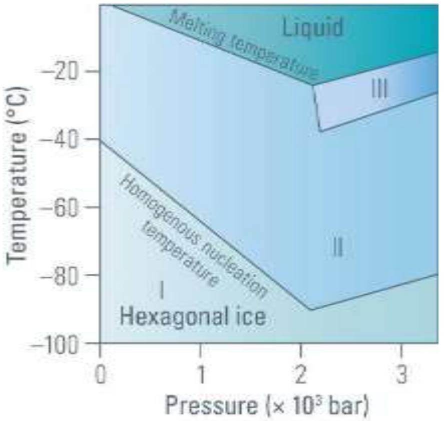






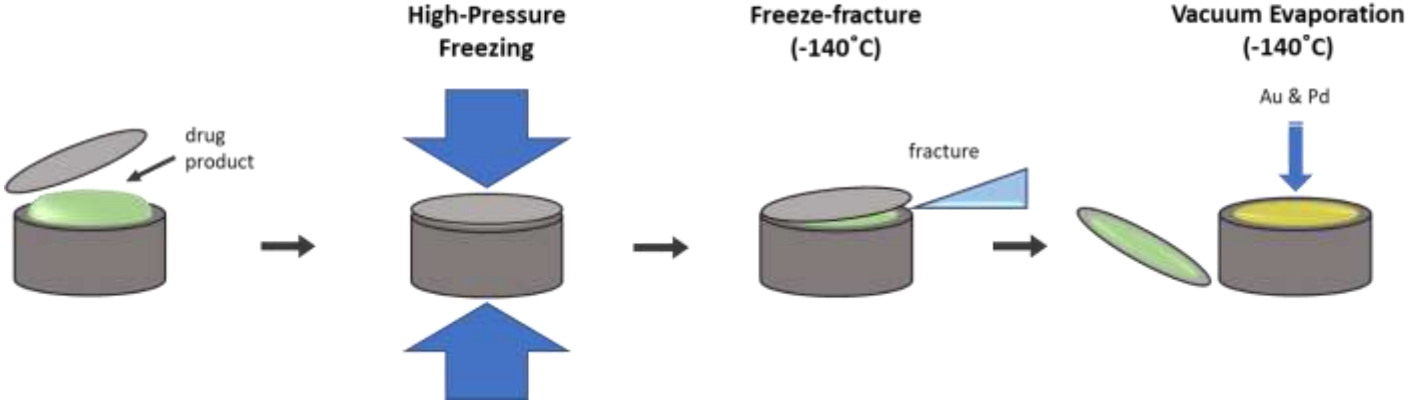


High-Pressure Freezing

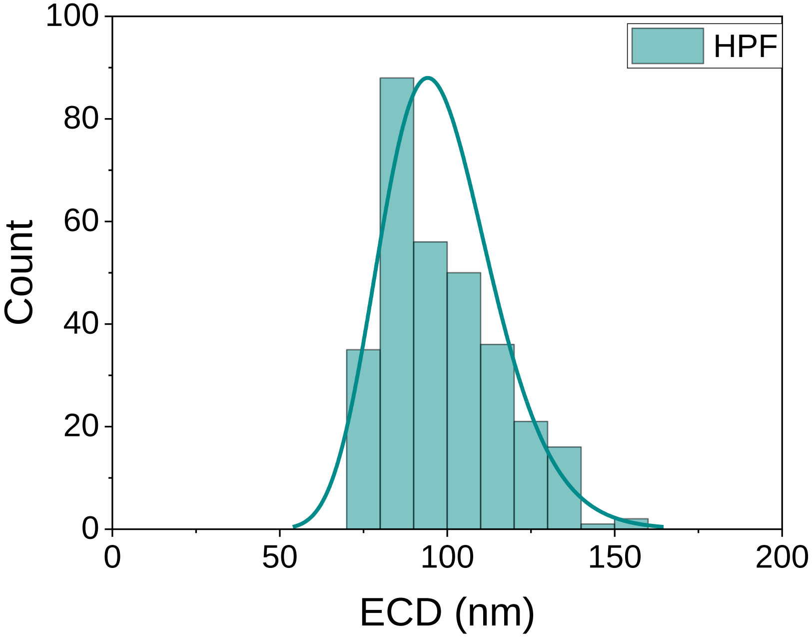


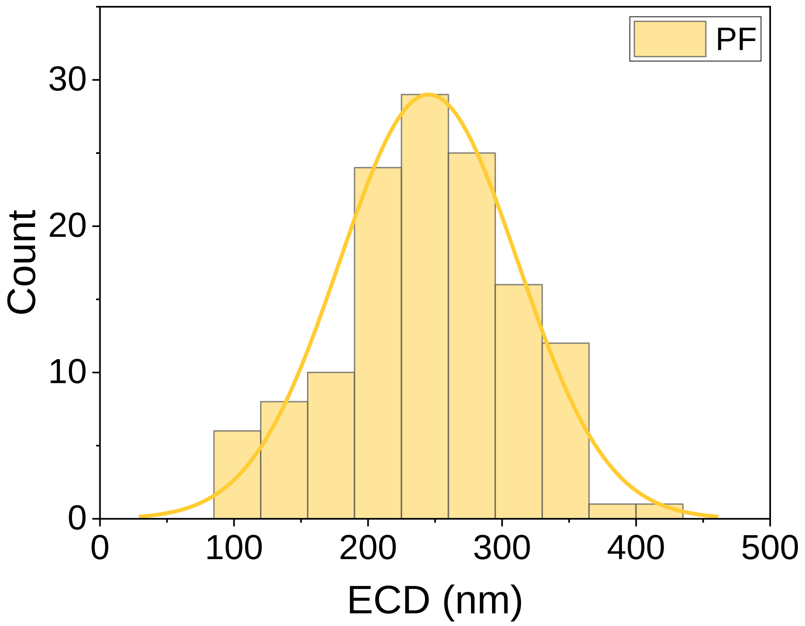
Kanno, H.; Speedy, R.; Angell, C., Supercooling of water to-92 C under pressure. *Science* **1975,** *189* (4206), 880-881.

- At 2 kbar homogeneous nucleation temperature is lowered
- Crystallization depends on cooling rate
- Synchronized pressurization and cooling

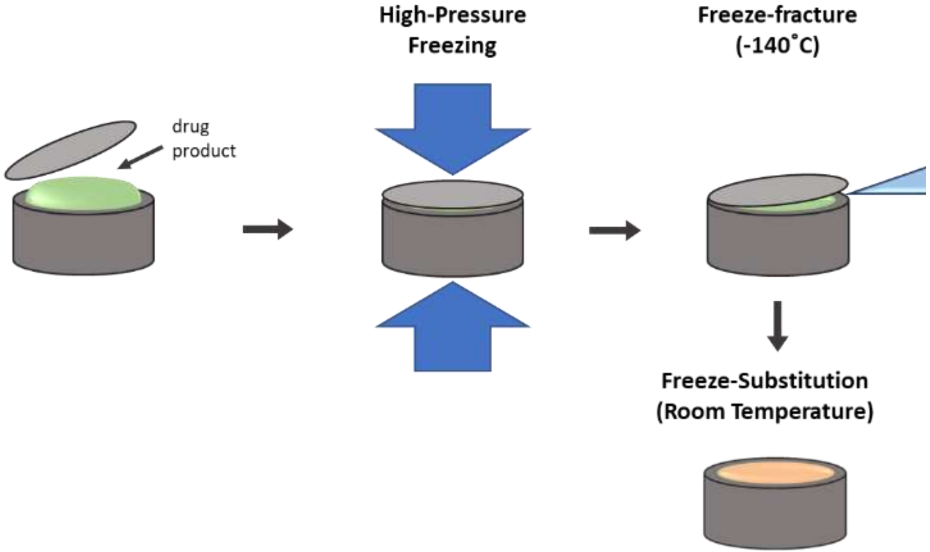


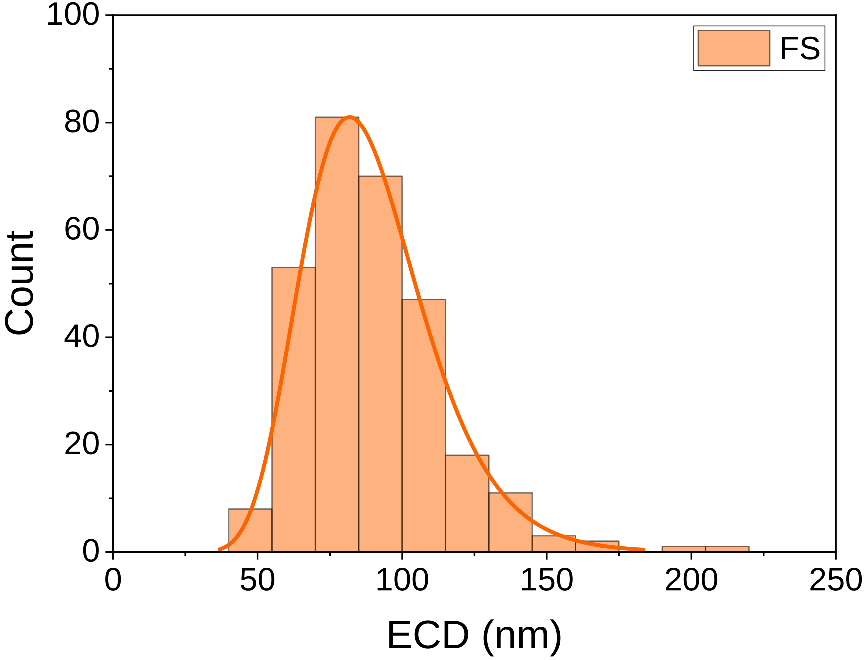
High-Pressure vs Plunge Freezing



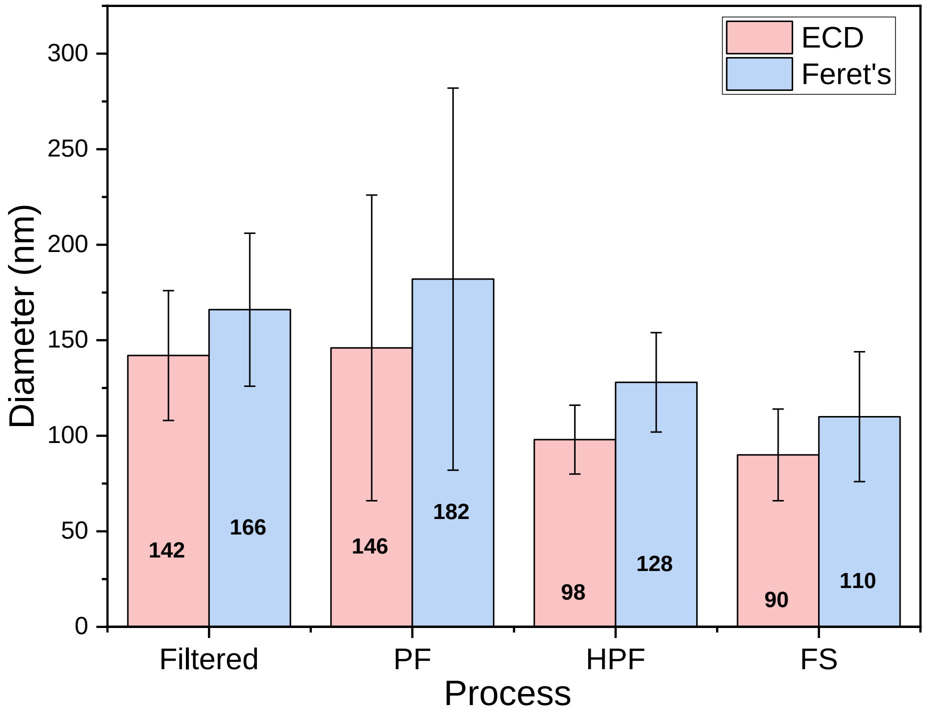


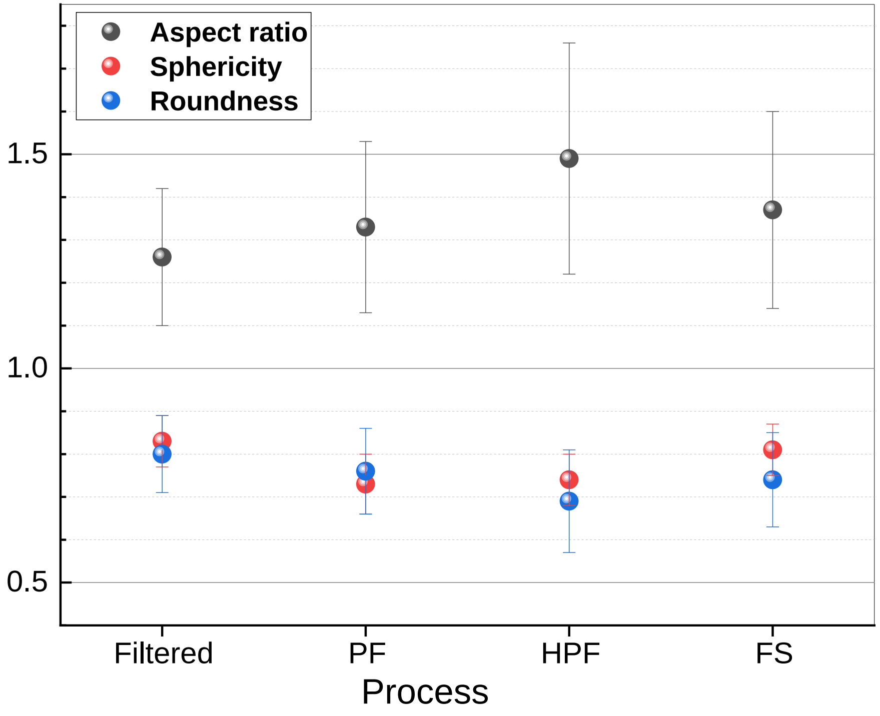
Freeze Substitution



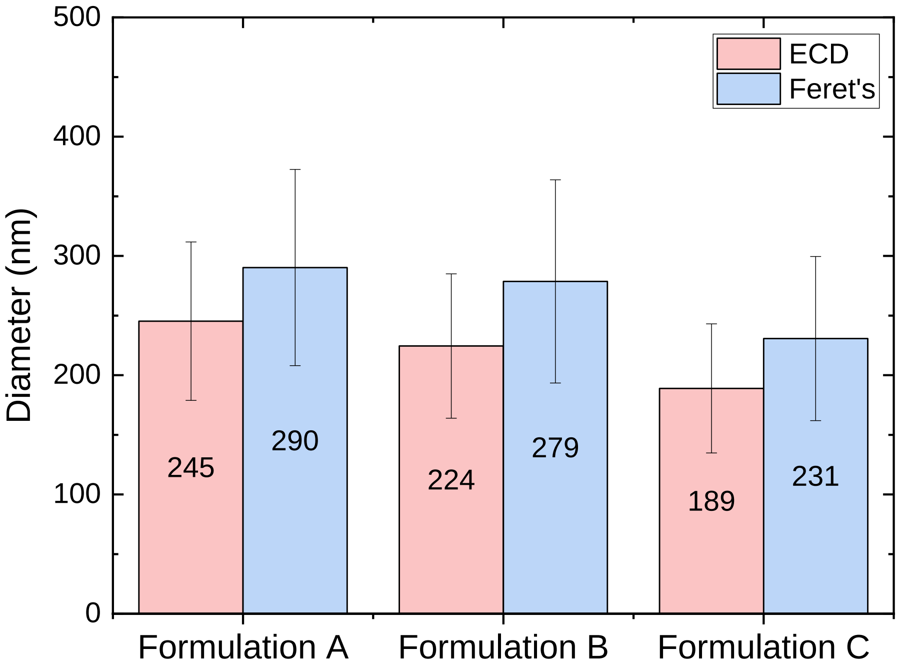


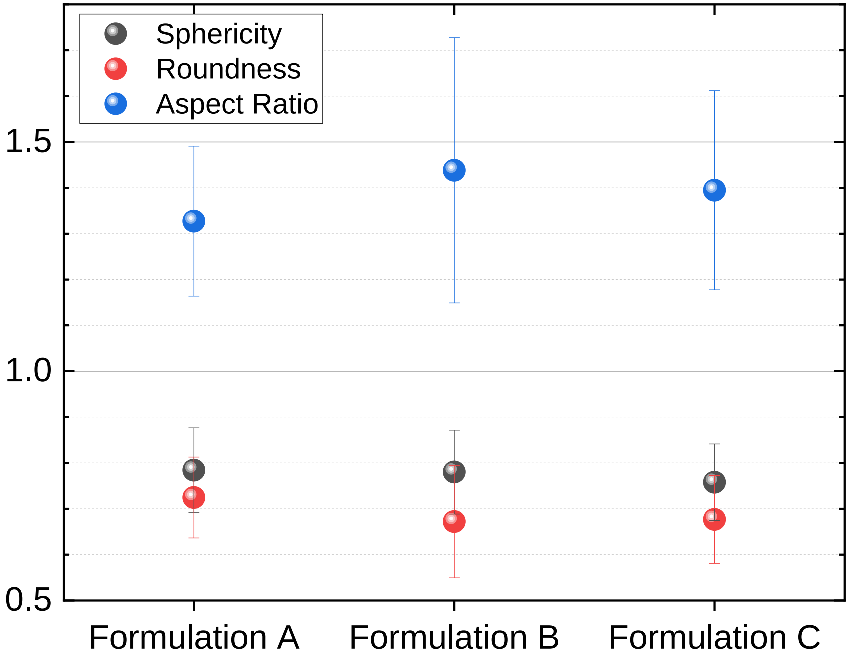
Cryofixation Strategies



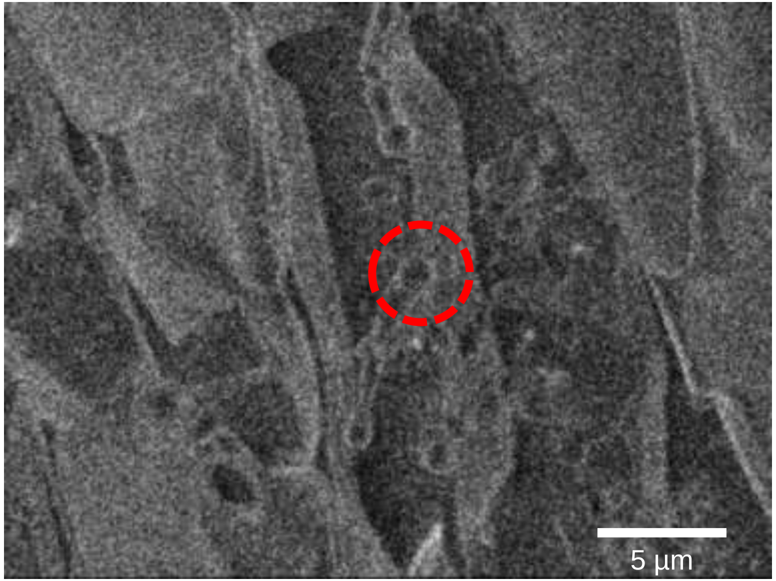


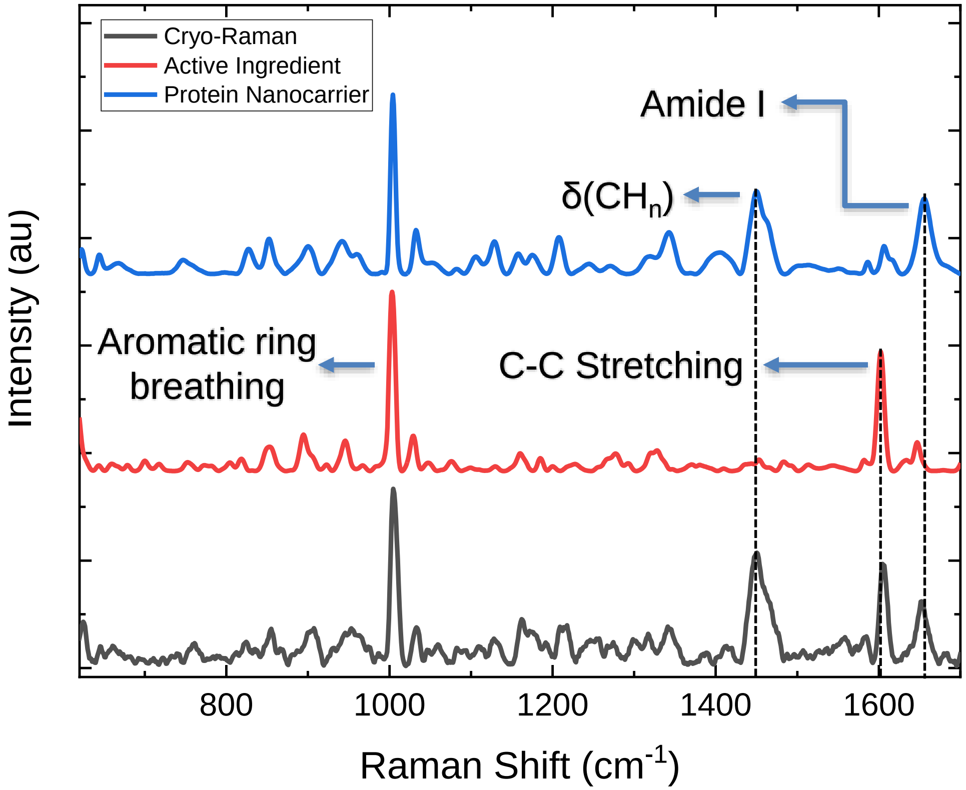
Comparing Formulations





Multi-Attribute Characterization





Challenge Question #2

Which sample preparation method will minimize artefacts in cryo-EM analysis of an aqueous suspension nanomedicine?

A. Chemical Fixation

B. High Pressure Freezing

C. Plunge Freezing

D. Filtering and drying

Summary

- Nanotechnology has been revolutionizing medicine
- Adequacy of in-vitro characterization methods; method development for particle size analysis, commonly used (light scattering) methods and their limitations
- Image based quantitative analysis via cryo-EM; cryofixation strategies, direct measurement of size and shape
- Potential for multi-attribute characterization in native-like state (cryo- or chemical fixation)

Acknowledgements

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