Lessons Learned from Nanomaterial Characterization: Critical Quality Attributes that Influence Biological Properties

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Disclaimer: The views expressed are of the presenter and should not be considered as the official position or policy of US FDA.

Conflict of Interest Statement

- No conflicts to declare

Objectives

- To showcase the importance of monitoring various physico-chemical attributes of nanomaterial to understand their interactions and impact on biological systems

Food and Drug Administration Mission

The FDA is responsible for

- Protecting the public health
  - by assuring the safety, efficacy, and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation.
- Advancing the public health by helping to speed innovations
  - that make medicines and foods more effective, safer, and more affordable; and helping the public get the accurate, science-based information they need to use medicines and foods to improve their health.

25% of US domestic spending is on products regulated by FDA


Food


Drugs & Biologics


Devices

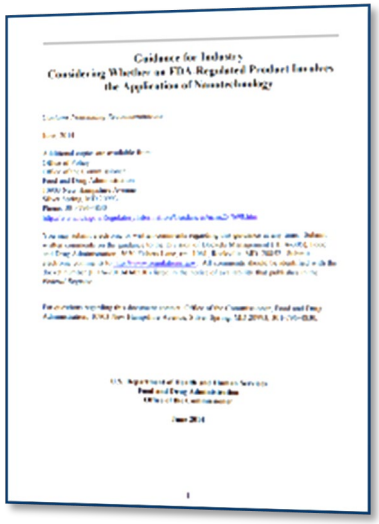

Cosmetics

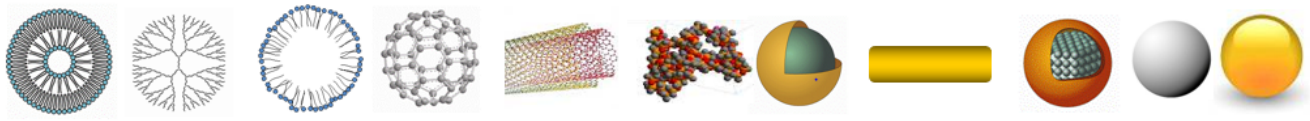

Veterinary


OTC Products

FDA Guidance on Whether an FDA-Regulated Product Involves the Application of Nanotechnology

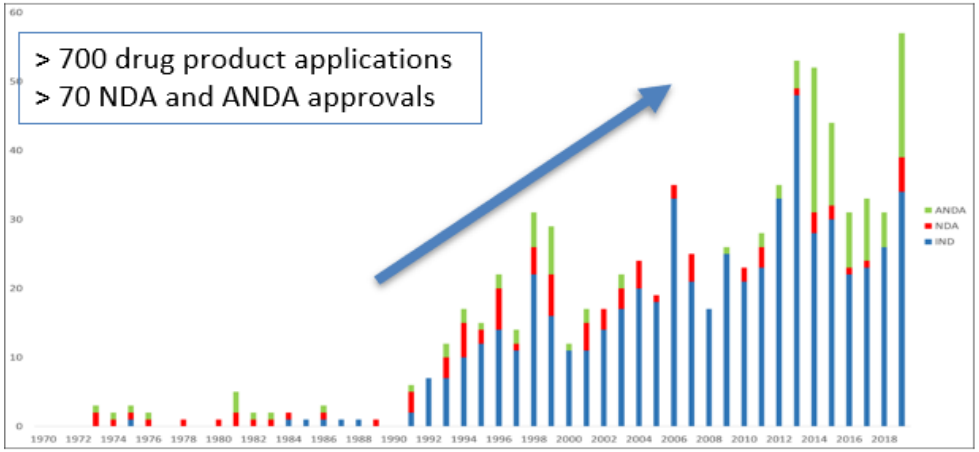
- No regulatory definition of nanotechnology or related terms
- FDA regulatory framework and review processes adequately identify and manage potential risks associated with the use of nanomaterials in products
- **Points to Consider**
  - Whether a material or end product is engineered to have at least one external dimension, or an internal or surface structure, in the nanoscale range (approximately 1 nm to 100 nm)
  - Whether a material or end product is engineered to exhibit properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer (1,000 nm)


Guidance for Industry
Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology



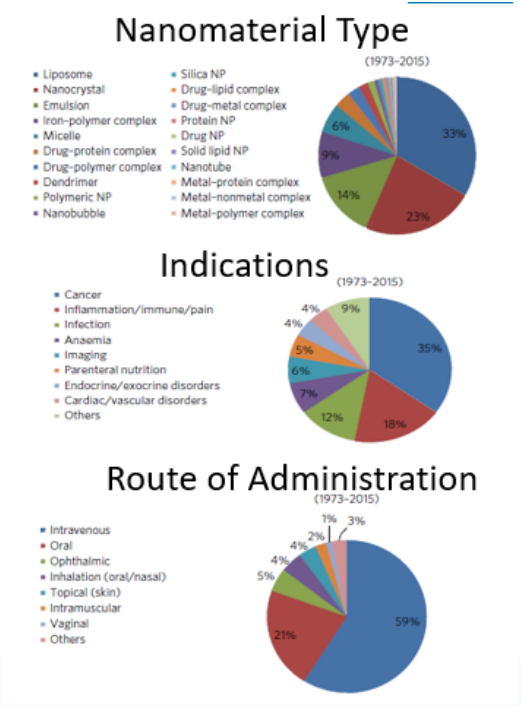
http://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm

Human Drug Product Submissions to FDA Containing Nanomaterials



Human drug product submissions to FDA containing nanomaterials between 1970-2019.

- Evolution from simpler drug delivery systems to highly complex, multicomponent, multifunctional structures and devices
- FDA is flexible to support innovation by promoting beneficial nanotechnology product development



https://www.fda.gov/science-research/nanotechnology-programs-fda/nanotechnology-task-force
D'Mello, S. R. et. al. Nature Nanotechnology, 2017 DOI: 10.1038/NNANO.2017.67

FDA Issued Final Guidances and Draft Guidance Related to Nanotechnology

- Final Guidance for Industry: Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology
- Final Guidance for Industry: Safety of Nanomaterials in Cosmetics
- Final Guidance for Industry: Assessing the Effects of Significant Manufacturing Process Changes, Including Emerging Technologies, on the Safety and Regulatory Status of Food Ingredients and Food Contact Substances, Including Food Ingredients that are Color Additives
- Final Guidance for Industry: Use of Nanomaterials in Food for Animals
- Final Guidance for Industry - Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labeling Documentation
- Draft Guidance for Industry: Drug Products Including Biological Products that Containing Nanomaterials
- Other relevant product specific guidances

https://www.fda.gov/science-research/nanotechnology-programs-fda/nanotechnology-guidance-documents

Research Infrastructure: Opportunity for Agency-Wide and Inter-Agency Collaboration

Monitoring Critical Quality Attributes
Drug products Containing Nanomaterial

Nanomaterial properties
Size, polydispersity
Shape/Dimensions
Surface characteristics
Composition
Quality
Purity
Stability
Endotoxin/sterility

Drug component
Encapsulated/unencapsulated drug(s)
Drug release
Metabolites
Purity, stability

Targeting agents
Small molecules, Peptides, Antibodies

Instrumentation

- Dynamic Light Scattering
- Laser Diffraction
- Nanoparticle tracking
- Spectroscopy – UV-Vis, NMR, Infrared, Raman, Fluorescence
- Chromatography – HPLC with UV-Vis, Fluorescence, Charged Aerosol Detector, Evaporative Light Scattering Detector
- Size exclusion chromatography or Asymmetric flow or Centrifugal flow Field Flow Fractionation with Multi-angle Laser Light Scattering, DLS, Refractive Index, UV-Vis
- Analytical Ultracentrifugation
- Inductively-coupled Plasma Mass Spectrometry
- Gas Chromatography Mass Spectrometry
- Thermogravimetric analysis
- Differential scanning calorimetry
- Scanning Electron Microscopy
- Transmission Electron Microscopy
- Atomic Force Microscopy
- Quartz crystal microbalance
- X-ray diffraction, X-ray fluorescence

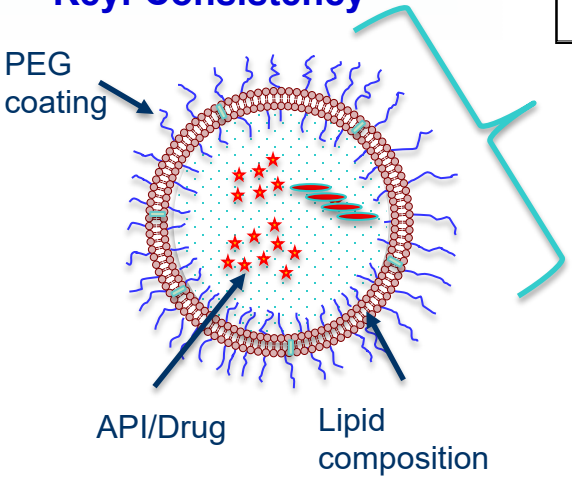
Nanoparticle Sample Preparation for *In Vitro* Studies

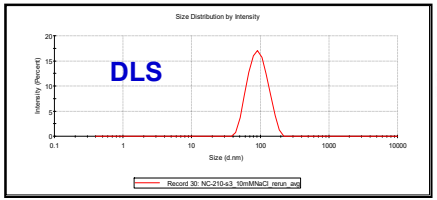
- Most metal and metal oxide nanoparticles can stay in aqueous suspension as colloids
- Some are insoluble and others will aggregate, agglomerate, or crash out of solution at higher ionic strength buffers, including cell culture media
- Protein or organic coatings may temporarily provide stability, suspendability, but these "coated particles" interact differently compared to "uncoated particles" *in vitro* and *in vivo*
- Organic non-covalent coatings may be easily removed become unstable in cell culture media, causing particles to aggregate/agglomerate
- Adequate characterization of particles in suspension is necessary under appropriate buffer conditions and monitors for change in size, agglomeration state, as a function of ionic strength, pH, and other factors
- The interactions of smaller particles with cells, their internalization, may be different from larger agglomerates

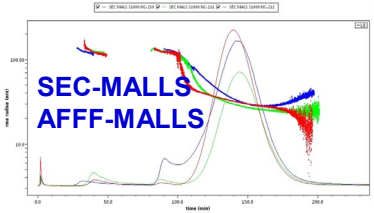
Liposome Characterization Case Study

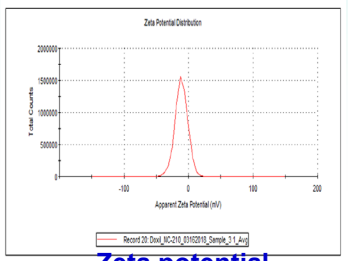
Size Distribution

Synthesis
Various Methodologies
Key: Consistency

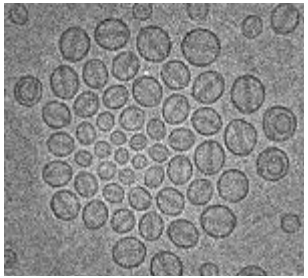




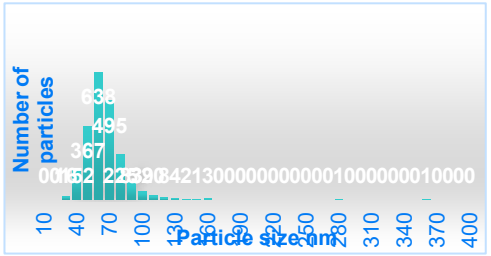


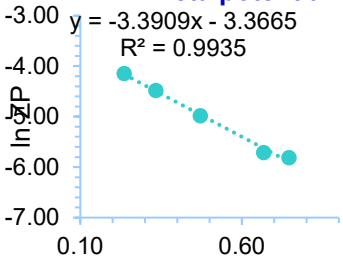


Zeta potential



Cryo-TEM

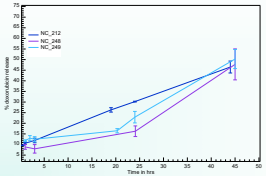




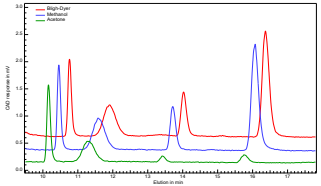
Fixed Aqueous Layer Thickness FALT

Additional methods:
Nanoparticle Tracking
Laser diffraction

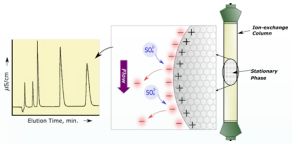
Drug Release Profile



Total, Encapsulated, Unencapsulated drug
LC-MS



HPLC CAD or ELSD or MS for lipid quantiation

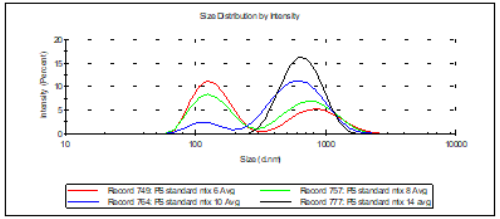


Intra and extravesicular salt concentration
Ion Chromatography

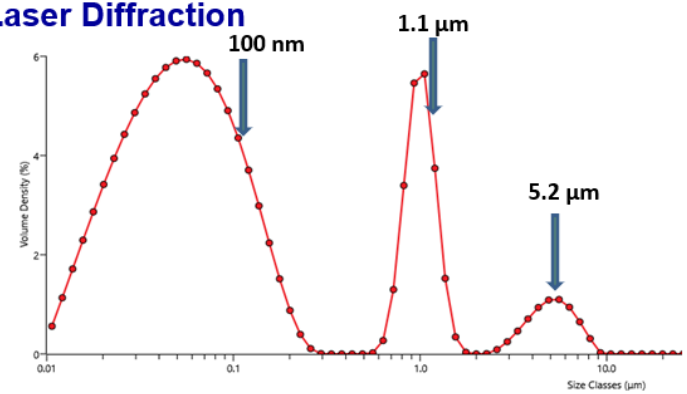
Measurement in Pristine Aqueous Suspensions

Principle	Type of measurement	Size range
Dynamic Light Scattering (DLS)	Hydrodynamic size	1 nm – 10 µm
Laser Diffraction (LD)	Hydrodynamic size	10 nm – 10 mm
Nanoparticle Tracking Analysis (NTA)	Hydrodynamic size	10 nm – 1 µm
Asymmetric Field-Flow Fractionation/Multi-angle Light Scattering	Hydrodynamic size & RMS Radius	3nm – 1 µm

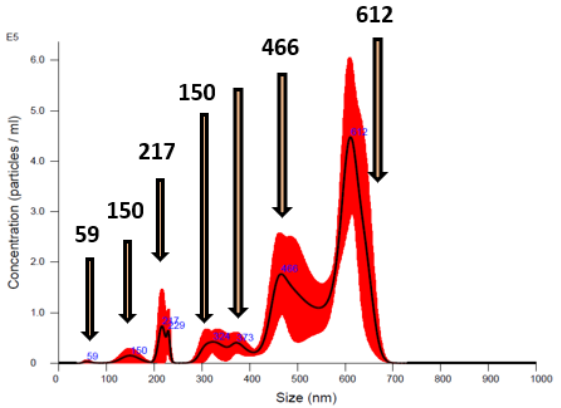
Dynamic Light Scattering (DLS)



Laser Diffraction

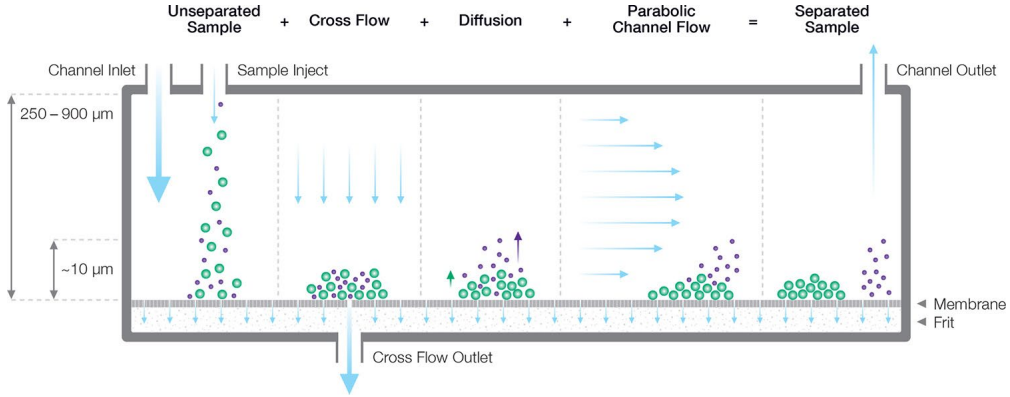


Nanoparticle Tracking Analysis



Several limitations for unknown samples and complex matrices

Fractionation of Mixtures: Asymmetric and Centrifugal Field Flow Fractionation (AFFF & CFFF)

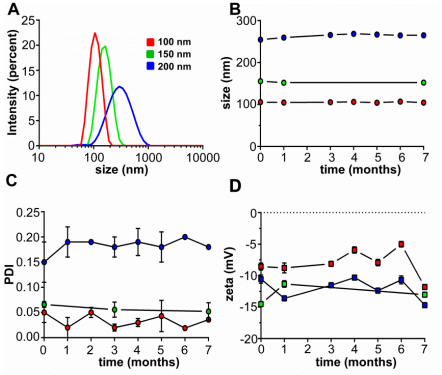


- Separation of particles based on hydrodynamic size and detection of size, composition through multiple online detectors
- Both hydrodynamic size and RMS radius can be measured simultaneously using on-line mode.
- Better resolution of particles within a narrow size range window.
- No stationary phase (column)
- Samples gets diluted and possibility of particles to stick to the membrane
- Upper size limit is ~ 1 µm

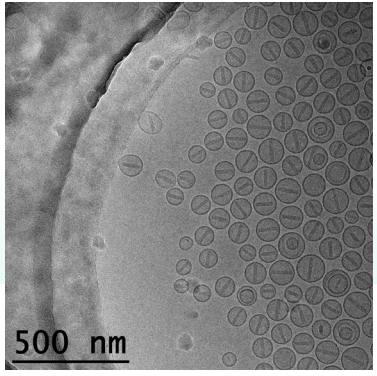
Size Effects on Drug Pharmacokinetics and Biodistribution

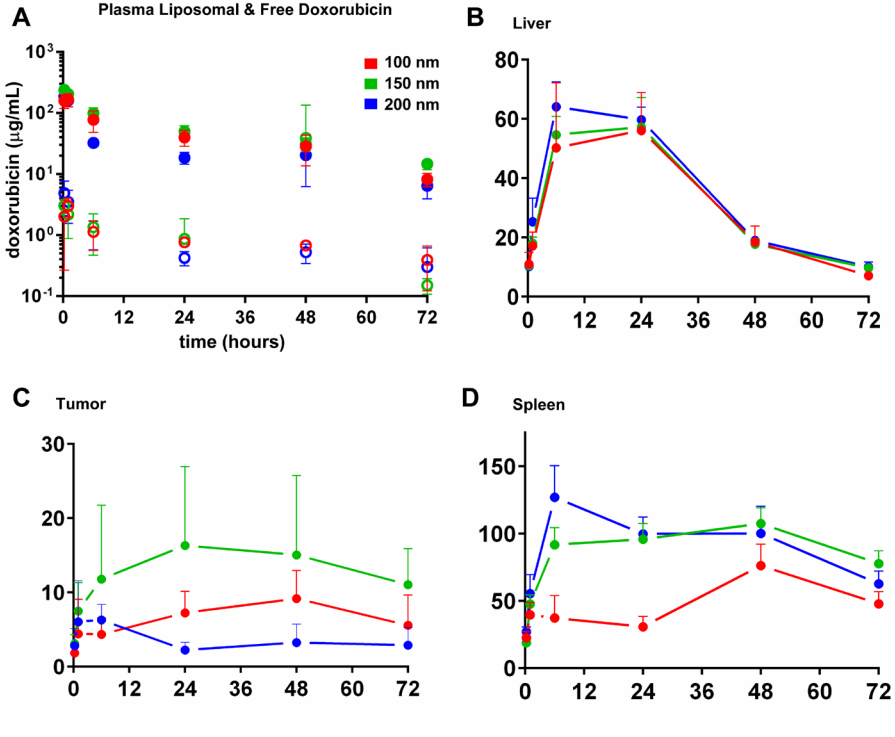
Example: 100, 150, and 200 nm Nominal Size Liposomal Doxorubicin

Size measurement - DLS



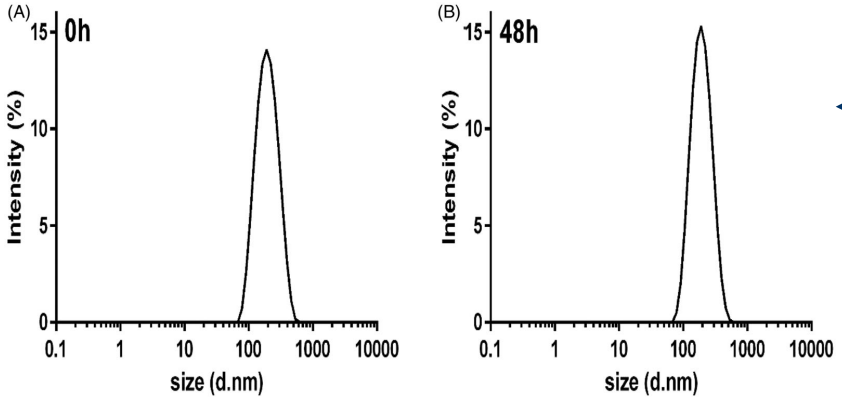
Size measurement - CryoTEM





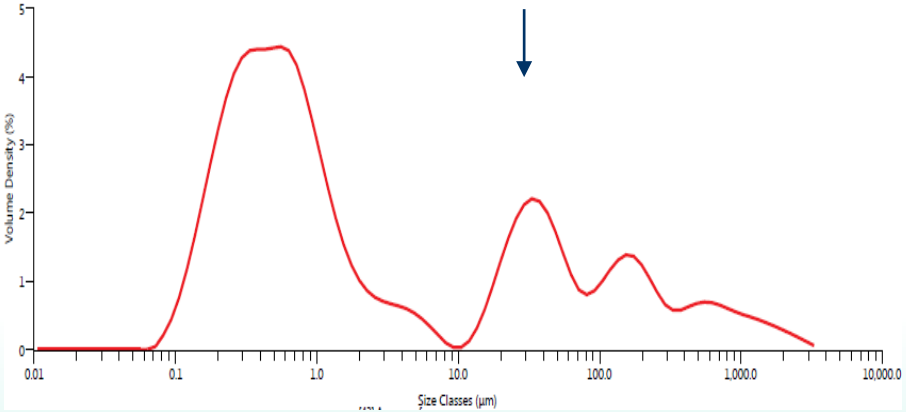
While drug PK data may be identical for different sizes, tissue biodistribution can be significantly altered

TiO_2 Nanoparticle Measurements

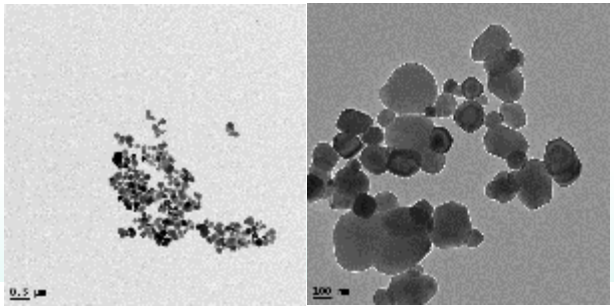


NIST P25 TiO2 Standard
Dynamic Light Scattering
Monodisperse upon sonication

Laser diffraction



Transmission Electron Micrographs

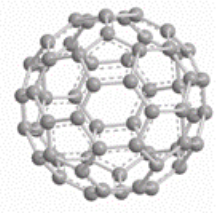


Food Grade TiO2

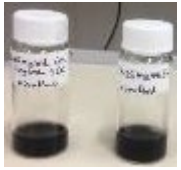
Pogribna, M. et. al. Nanotoxicology, 2020, 14:4, 534-553, DOI:10.1080/17435390.2020.1723730

Challenges in Sample Preparation for *In Vitro* Studies–Graphene Case Study

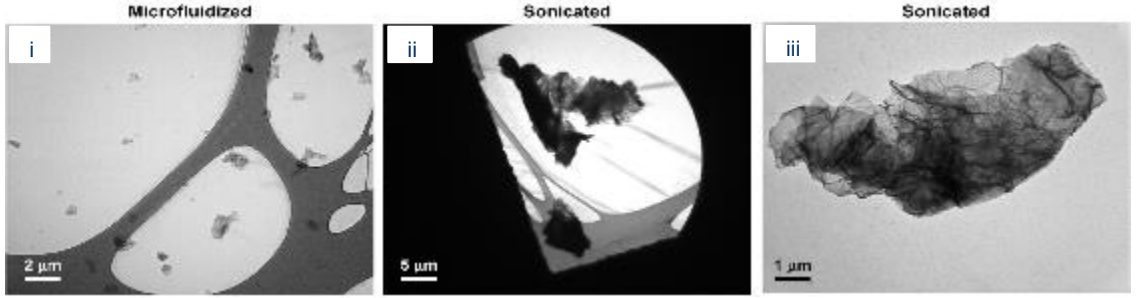
- Carbonaceous material such as carbon nanotubes, fullerenes and graphene are insoluble in water
- How do you suspend them in cell culture media for in vitro studies?
- Do different dispersions have different biological effects based on size and agglomeration?



Sonicated

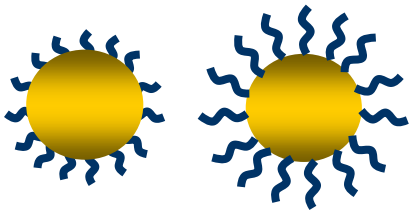


Microfluidized

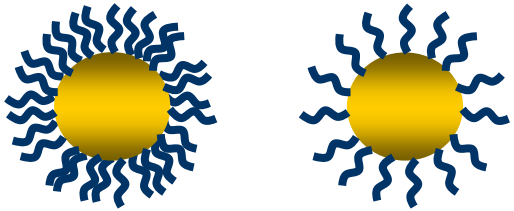


Surface Coatings Influence Biological Interaction

- Molecular weight of coating moiety is important.
  - Protein binding
  - Stability
  - Toxicity
  - Efficacy



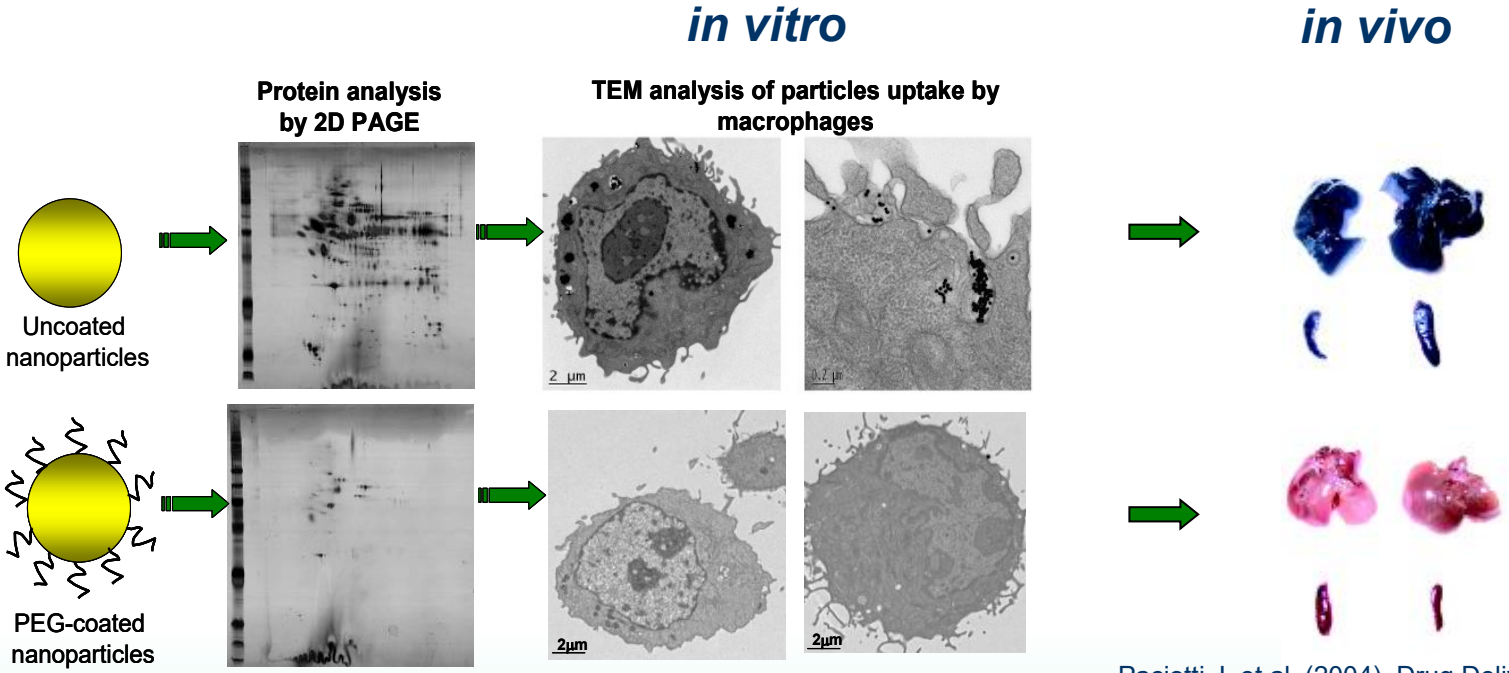
- Density of coating moiety and stability is critical.
  - Differential protein binding
  - Opsonization/immune system recognition
  - Biodistribution
  - Toxicity
  - Efficacy



- Many common coating polymers are not UV-Vis sensitive.

Is the coating Covalent or Non-covalent?
What is the stability of the coating (e.g., organic polymer)?

Presence or Absence of Surface Coatings Can Alter Biodistribution



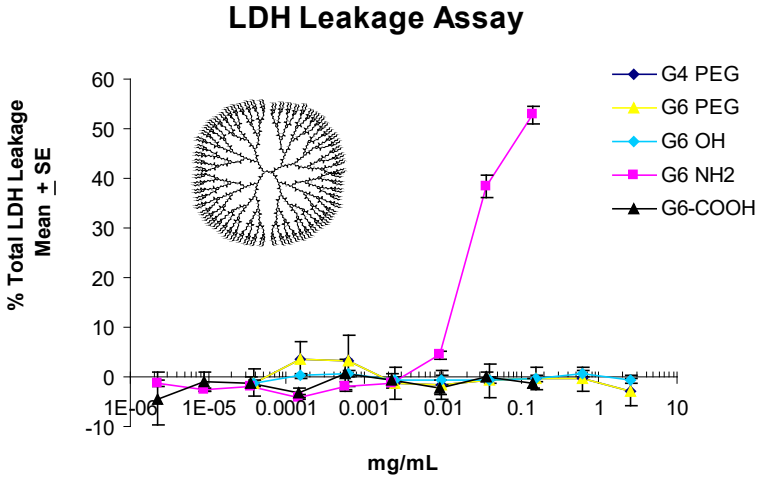
Dobrovolskaia et al., (2008), Mol.Pharm., 5:487-495.

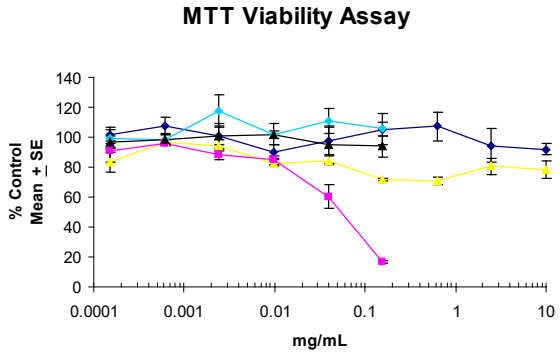
Paciotti J. et al.,(2004), Drug Delivery,11:169-183.

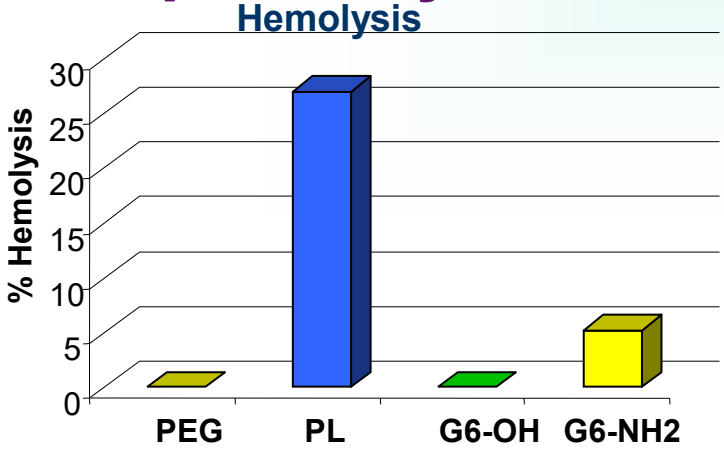
Difference in surface characteristics can cause dramatically different *in vivo* outcomes.

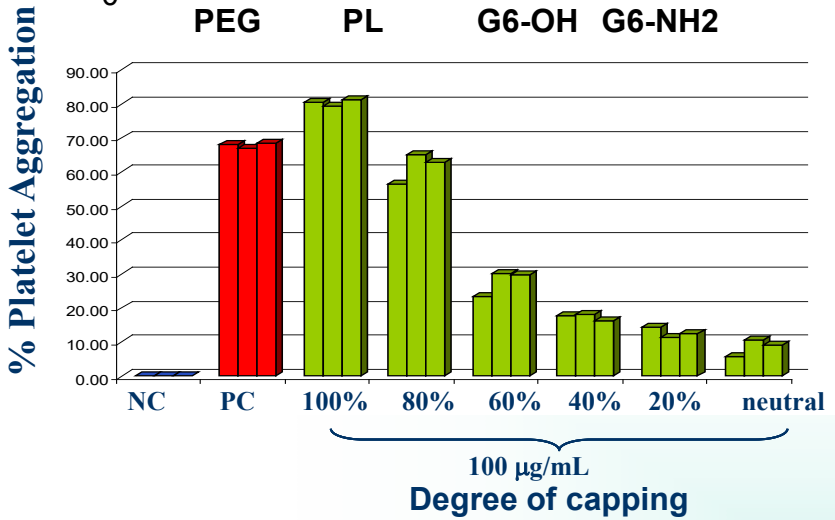
Surface Characteristics and Biocompatibility

Dendrimers





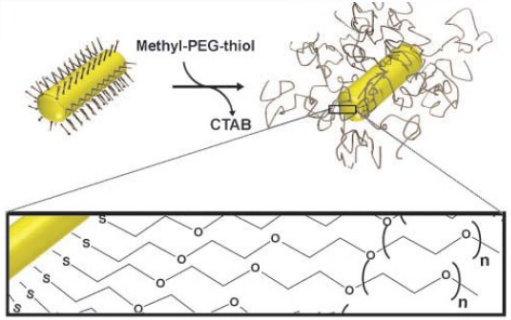




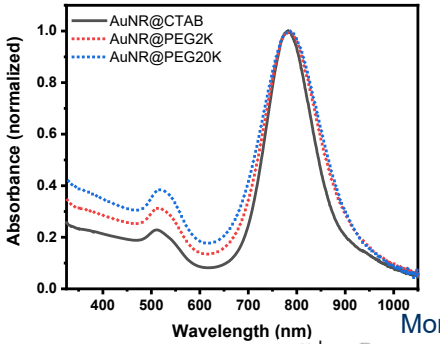
Surface characteristics are important for biocompatibility and can be modulated

Impurities Assessment in the Test Article

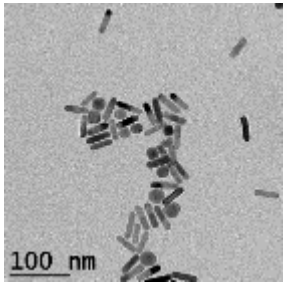
Gold Nanorods case study

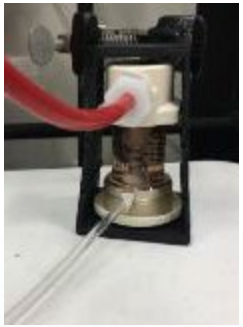


UV-Vis

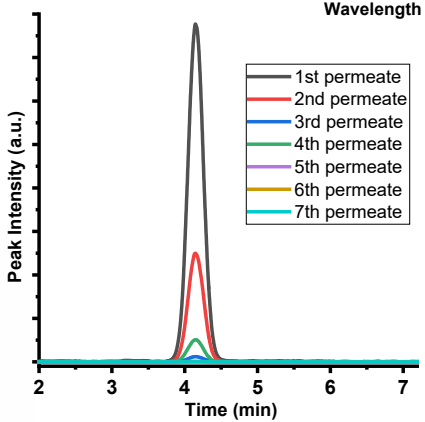


TEM



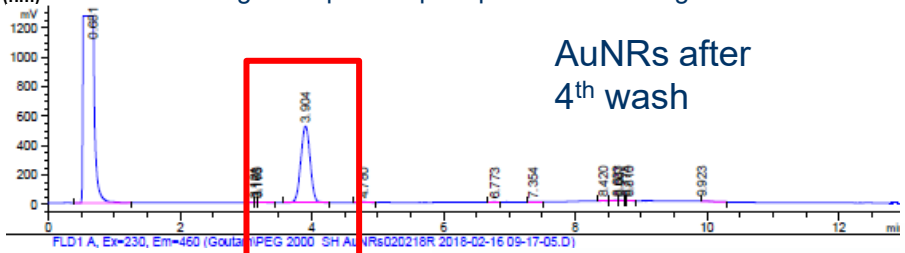


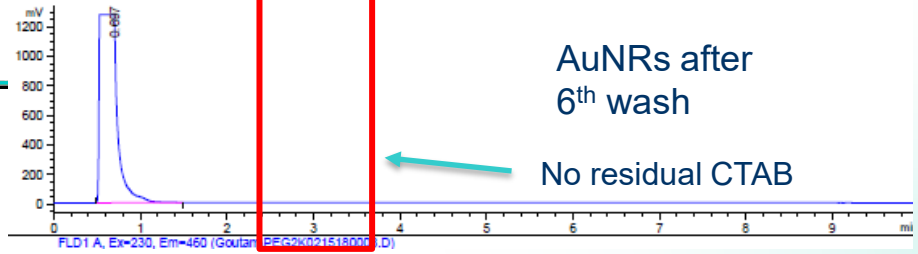
Stirred cell Purification



HPLC-ELSD of CTAB

Monitoring for impurities post-purification through HPLC-ELSD

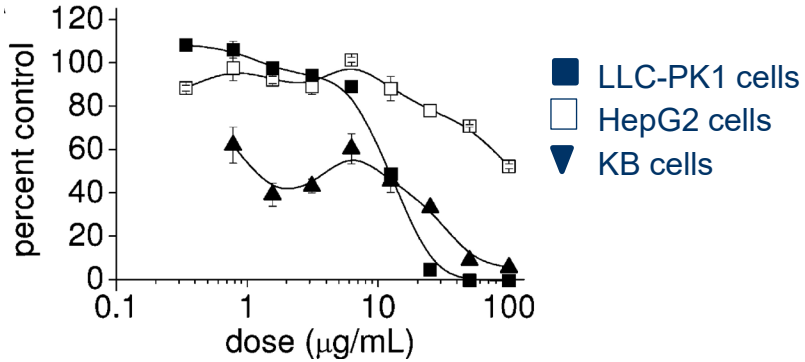




Appropriate methods for detection/quantitation of impurities must be employed

Impurities/Surfactants Cause Cytotoxicity

Gold Nanorods test article
MTT cell viability assay

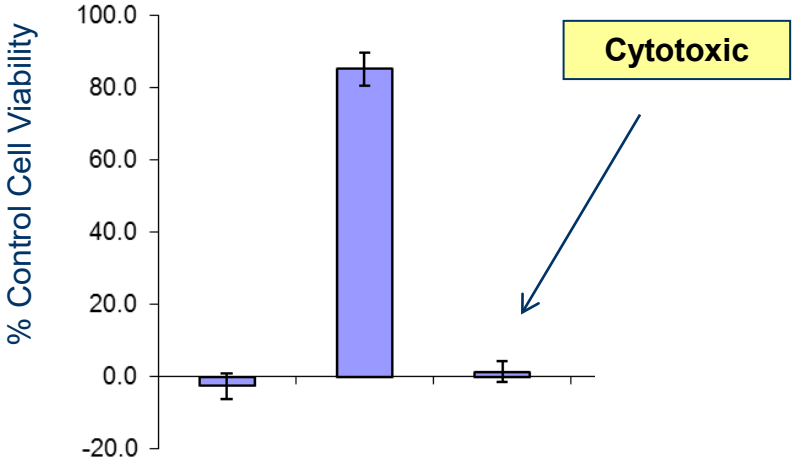


Cytotoxicity of PEG coated gold nanorods

Ultrafiltration

Gold nanorod sample →

LLC-PK1 MTT Cytotoxicity Assay



Cytotoxicity is attributed to the filtrate,
which contained residual CTAB surfactant as impurity

- Cytotoxicity was associated with the buffer, not particles
- Impurities/residual chemicals from synthesis need to be monitored and quantified with appropriate assays

Alexei P. Leonov et. Al.; ACS Nano 2008, 2, 2481-2488.

Summary

- Significant increase in product submissions containing nanomaterial regulated by the FDA–from simple to complex systems
- Knowledge about nanomaterial evolved in the past two decades with science advances
- Many nanomaterial-specific properties influence biological interaction and alter safety
- Advanced understanding of these properties and nuanced differences would enable better products to market

Acknowledgments

- NCTR-ORA Nanotechnology Core Facility Staff
- Nanotechnology Task Force, Office of the Commissioner
- National Toxicology Program, NIEHS/NIH

Current members:

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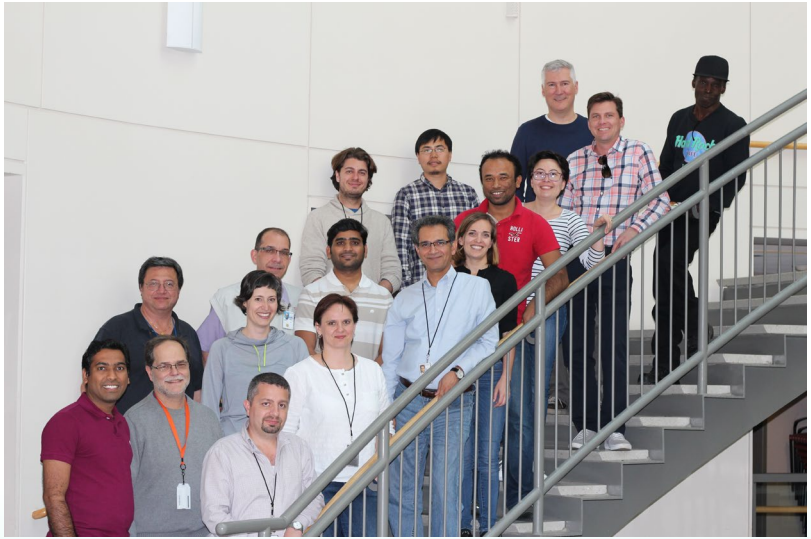
Angel Paredes, PhD
Tariq Fahmi, MD, PhD
Nathan Koonce, PhD
Goutam Palui, PhD
Sanghamitra Majumdar, PhD
Arjun Sharmah, PhD
Achyut Raghavendra, PhD
Kevin Trout, PhD
Sungyoon Jung, PhD
Brandon Canup, PhD
Anne D'Achille, PhD
Kal'e Carlisle
Jessie Collins



Past members:

Paul Howard, PhD
Udaya Nasini, PhD
Sunil Ramasahayam, PhD
Suresh Dadiboyena, PhD
Trever Todd, PhD
Ioana-Mihaele Popescu, DVM, PhD
Xian Wu, PhD
Taylor Ingle, PhD
Alokita Karmakar, PhD
Yongbin Zhang, DVM, PhD
Jia Yao, PhD
Melissa Collins, PhD
Suman Ghorai, PhD
Aahana Ganguly, PhD
Ammu Mathews, PhD
William Monroe
Yvonne Jones