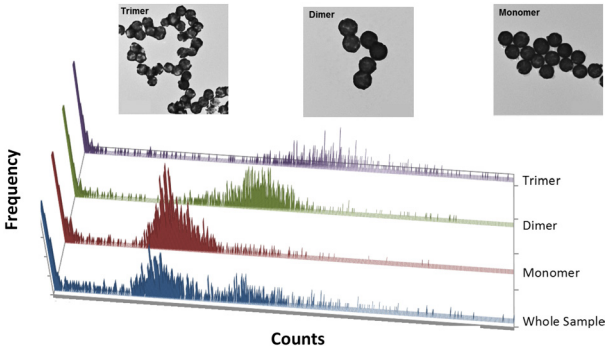
NCL Nanotechnology Characterization Laboratory

Parameters, Methods and Considerations for the

Physicochemical Characterization of Colloidal Metal Nanoparticles

Size/Size Distribution

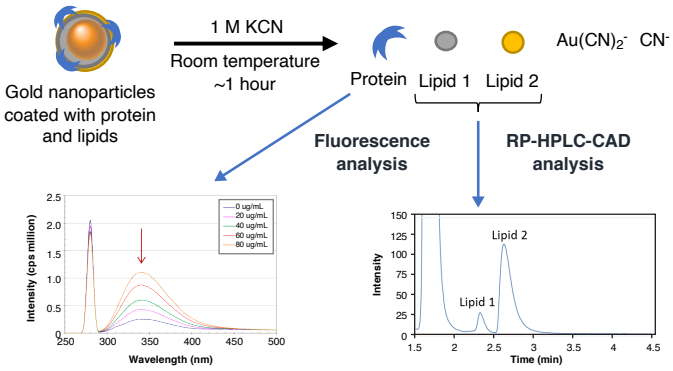
- Dynamic light scattering (DLS)
- Multi-angle light scattering (MALS)
- Laser diffraction
- Transmission electron microscopy (TEM)
- Resistive pulse sensing
- Asymmetric-flow field-flow fractionation (AF4) – MALS/DLS
- Single particle inductively-coupled plasma mass spectrometry (spICP-MS)



Single particle ICP-MS and representative TEM images of PEGylated core-shell nanoparticles. spICP-MS can resolve the different species in a single run and determine the size of each population.

Composition

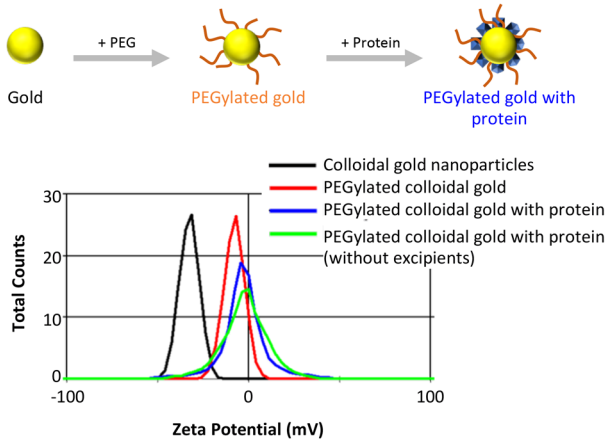
- Metal concentration: total & free
- Coating (polymer, protein, lipid) concentration: total & free
- Targeting ligand concentration: total & free
- Excipient concentrations
- Particles per mL concentration
- Osmolality, viscosity measurements



Colloidal gold nanoparticles are dissolved using KCN to afford free protein and lipid surface ligands. The resulting solution is analyzed for concentration of individual components.

Surface Characteristics

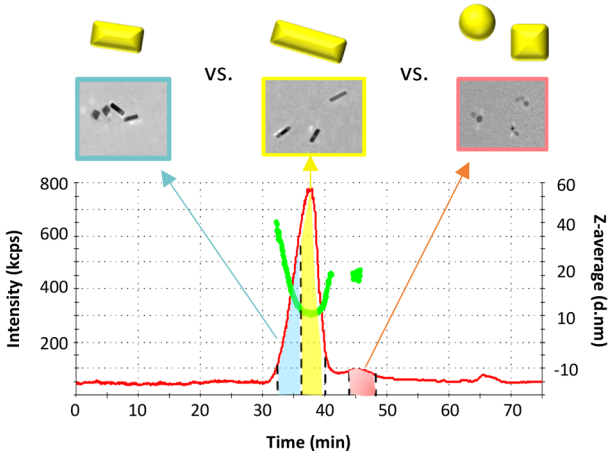
- Zeta potential
- Protein binding assessment by AF4-MALS/DLS
- Quartz crystal microbalance with dissipation monitoring (QCM-D)



Monitoring the reaction progress of PEG and protein grafting to the surface of colloidal gold nanoparticles by zeta potential. Measurement conditions were identical for all samples.

Purity

- Metal impurities
- Shape distribution (e.g., spheres versus rods)
- Coating impurities
- Residual solvents and reagents



Purity assessment of PEGylated gold nanorods as defined by the presence of non-rod shaped particles. Particles were separated by AF4-DLS, and collected fractions were analyzed by TEM to confirm shape. Adapted from Anal Bioanal Chem, 2020, 412(2), 425-428.

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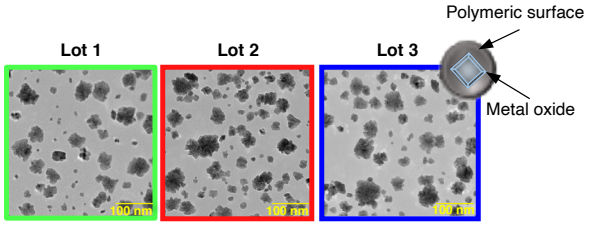
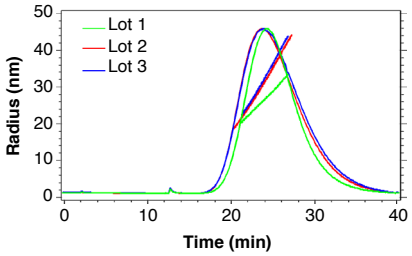
Batch-to-Batch Consistency

- Assessed by choosing relevant parameters (i.e., lot release criteria) that relate to a desired in vivo outcome



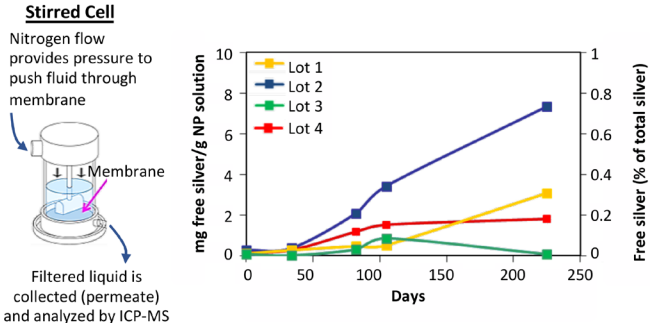


Flow-mode AF4-MALS could identify a difference between the three lots, whereas TEM could not.

Representative TEM images (top) versus flow-mode AF4-MALS (bottom) measurements for three batches of a polymer coated metal oxide formulation. In this case, AF4-MALS was better suited in assessing the size distribution and differences between these three lots. Adapted from J Control Release, 2019, 299, 31-43.

Stability

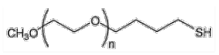
- Size/Size distribution; aggregation
- Free metal ion release
- Coating release
- Stability in plasma
- Solvent, thermal, pH, photo, freeze-thaw, lyophilization, centrifugation, filtration
- Storage conditions/shelf-Life

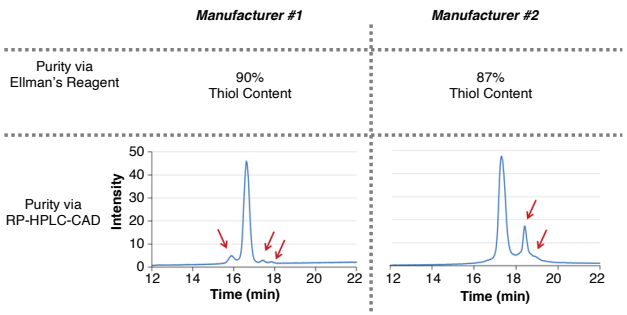


Stability assessment of four batches of colloidal silver nanoparticles as determined by free silver concentrations. Stirred cell filtration was used to separate free silver from nanoparticle silver. The permeate silver concentration was measured by ICP-MS.

Starting Material Characterization

- Colloidal metal NPs: size, shape, composition & purity
- Coating : molecular mass, polydispersity Index, purity, functionality
- Storage conditions/shelf-life

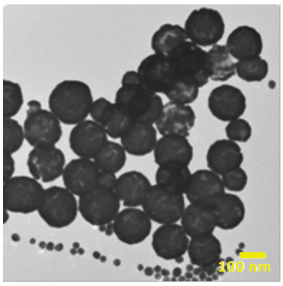


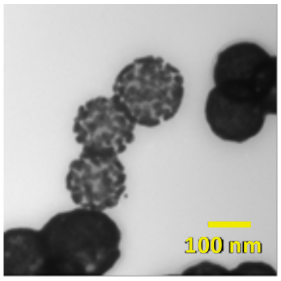


Purity and functionality characterization of 20 kDa mPEG-SH from two manufacturers. Purity was assessed by RP-HPLC-CAD and thiol content by the Ellman's reagent. Adapted from AAPS J, 2017, 19(1), 92-102.

Morphology

- Transmission electron microscopy (TEM)





Representative TEM images of gold-core silica-shell nanoparticles. In addition to determining the size distribution, TEM can be used to evaluate morphology and purity assessment. Here, smaller gold nanoparticles and incomplete gold coating were observed. Elemental composition was confirmed by energy dispersive spectroscopy (EDS).

Relevant NCL Publications

Anal Bioanal Chem, **2020**, 412(2), 425–428. PMID: 31776639
J Control Release, **2019**, 299, 31–43. PMID: 30797868
Pharmaceutical Research, **2019**, 37, 6. PMID: 31828540
Methods in Molecular Biology, Vol. 1628, **2018**, p. 37–47. PMID: 29039091
Methods in Molecular Biology, Vol. 1628, **2018**, p. 49–55. PMID: 29039092
Methods in Molecular Biology, Vol. 1628, **2018**, p. 57–63. PMID: 29039093
Anal Bioanal Chem, **2017**, 409(24), 5779–5787. PMID: 28762066

About NCL

The Nanotechnology Characterization Laboratory (NCL) is a resource for nanotech researchers and organizations developing nano-based therapies and diagnostics. The NCL provides preclinical characterization services through various collaboration mechanisms. Learn more by visiting our website: https://ncl.cancer.gov

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v2, DOI: 10.17917/C73011