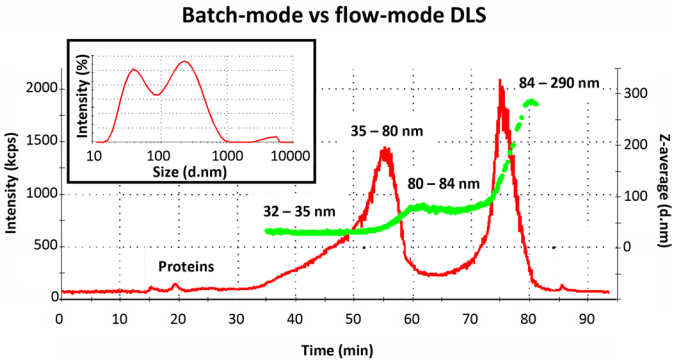
NCL Nanotechnology Characterization Laboratory

Parameters, Methods and Considerations for the Physicochemical Characterization of Liposomal Products

Size/Size Distribution

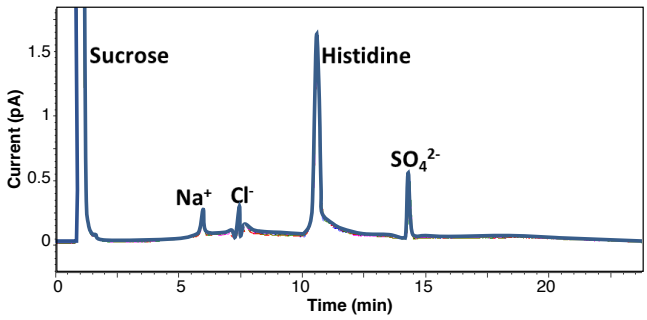
- Dynamic light scattering (DLS)
- Multi-angle light scattering (MALS)
- Laser diffraction
- Cryogenic-transmission electron microscopy (Cryo-TEM)
- Resistive pulse sensing
- Asymmetric-flow field-flow fractionation (AF4) – MALS/DLS



Batch-mode (inset) versus flow-mode DLS measurements of dual-drug loaded liposomes. Multiple size populations are observed by both techniques and indicate a polydispersed sample. However, flow-mode DLS can better resolve the size distribution of each population. Adapted from Anal Bioanal Chem, 2020, 412(2), 425-428.

Composition

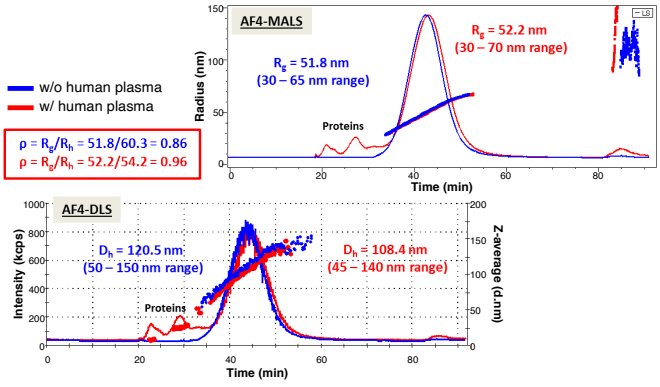
- Drug concentration: total, free & encapsulated
- Drug distribution as a function of size
- Targeting ligand concentration: total, bound & unbound
- Individual lipid concentrations
- Counterion concentrations: interior & exterior
- Excipient concentrations
- Particles per mL concentration
- Osmolality, viscosity measurements



Counterion and excipient concentrations for PEGylated liposomal doxorubicin measured by RP-HPLC with charged aerosol detection (CAD). Adapted from J Pharm Biomed Anal, 2019, 165, 41-46.

Surface Characteristics

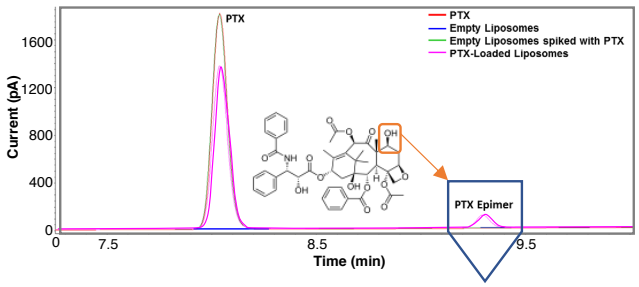
- Zeta potential
- Protein binding assessment by AF4-MALS/DLS
- Quartz crystal microbalance with dissipation monitoring (QCM-D)



Flow-mode AF4-MALS (top) and AF4-DLS (bottom) of PEGylated irinotecan liposomes before and after incubation in human plasma. The increase in the ratio (ρ) of the measured MALS (R_g) and DLS (R_h) sizes after human plasma incubation suggests protein binding to the surface of the liposomes. Adapted from Anal Bioanal Chem, 2020, 412(2), 425-428.

Purity

- Drug impurities
- Lipid impurities
- Free drug/lipid/targeting ligand concentrations
- Residual solvents and reagents

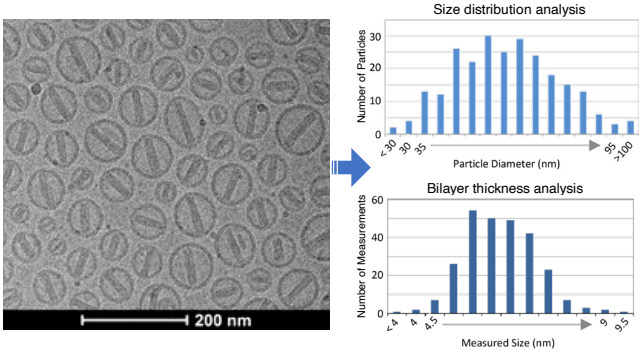


Analyzed by RP-HPLC and mass spectrometry

Purity assessment of PEGylated liposomal paclitaxel (PTX) as defined by the presence of drug impurities. The drug epimer concentration was measured by RP-HPLC and its identity confirmed by mass spectrometry.

Morphology

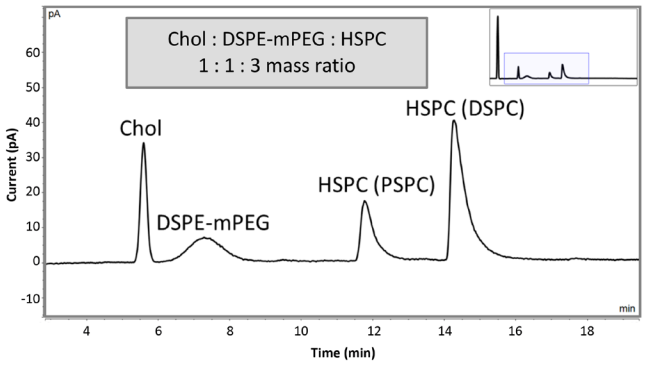
- Cryo-TEM can be used to evaluate:
  - Size distribution
  - Liposome morphology
  - Internal liposome volume
  - Bilayer thickness
  - Number of lamellae
  - Drug appearance/state



Representative cryo-TEM image of PEGylated liposomal doxorubicin. Cryo-TEM was used to determine size and morphology of the liposomes.

Starting Material Characterization

- Drug identity (structure)
- Drug purity (degradation products)
- Lipid composition (structure, fatty acid distribution)
- Lipid purity (free fatty acid, lysophospholipids)
- Storage conditions/shelf life



Lipid composition (identity and individual lipid concentrations) and purity (presence of free fatty acid and lysophospholipids) were determined by RP-HPLC with charged aerosol detection (CAD) for a commercially available lipid mix. The theoretical mass ratio was confirmed.

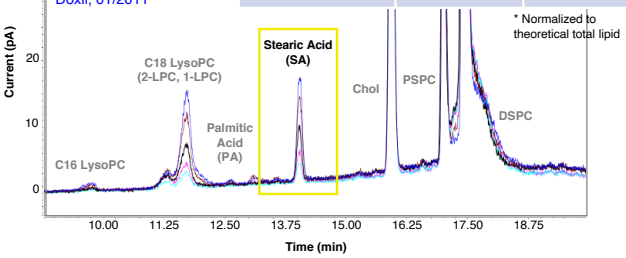
Relevant NCL Publications

Anal Bioanal Chem, **2020**, 412(2), 425–428. PMID: 31776639
J Control Release, **2019**, 299, 31–43. PMID: 30797868
J Pharm Biomed Anal, **2019**, 165, 41–46. PMID: 30502551
Pharmaceutical Research, **2019**, 37, 6. PMID: 31828540
Methods in Molecular Biology, Vol. 1628, **2018**, p. 49–55. PMID: 29039092
Anal Bioanal Chem, **2017**, 409(24), 5779–5787. PMID: 28762066

Stability

- Size/Size distribution; aggregation
- Drug leakage and degradation
- Hydrolysis of lipids
- Drug release in plasma
- Solvent, thermal, pH, photo, freeze-thaw, lyophilization, centrifugation, filtration
- Storage conditions/shelf-life

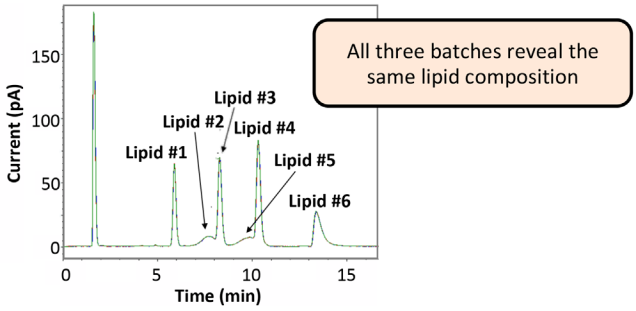
ID	Exp. Date	%Stearic Acid*
SunPharma	05/2018	0.65%
Doxil	05/2018	0.24%
Doxil	08/2015	1.8%
Doxil	04/2014	3.5%
Doxil	01/2011	4.5%



Stability assessment of PEGylated liposomal doxorubicin as defined by the hydrolysis of phospholipids. The formation of free fatty acids and lysophospholipids of several batches with varying expiration dates were measured by RP-HPLC with charged aerosol detection (CAD).

Batch-to-Batch Consistency

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



Batch-to-batch consistency for lipid nanoparticles with siRNA was assessed by quantitation of the lipid composition. Six individual lipid concentrations for three batches were determined by RP-HPLC with charged aerosol detection (CAD).

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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