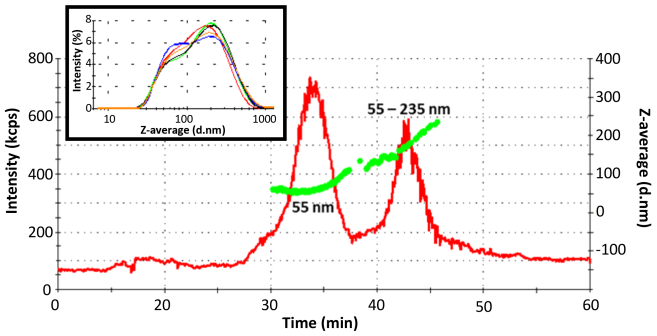
NCL Nanotechnology Characterization Laboratory

Parameters, Methods and Considerations for the Physicochemical Characterization of Polymeric 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



Batch-mode (inset) versus flow-mode DLS measurements of a micellar drug formulation. Multiple size populations are observed by both measurements and indicate a polydispersed sample. However, flow-mode DLS (coupled to AF4) can better measure the size distributions of each population. Adapted from Anal Bioanal Chem, 2020, 412(2), 425-428.

Composition

- Drug concentration: total, free & encapsulated
- Prodrug drug content
- Drug/Prodrug loading distribution (as a function of size)
- Targeting ligand concentration
- Individual polymer concentrations
- Excipient concentrations
- Particles per mL concentration
- Osmolality, viscosity measurements

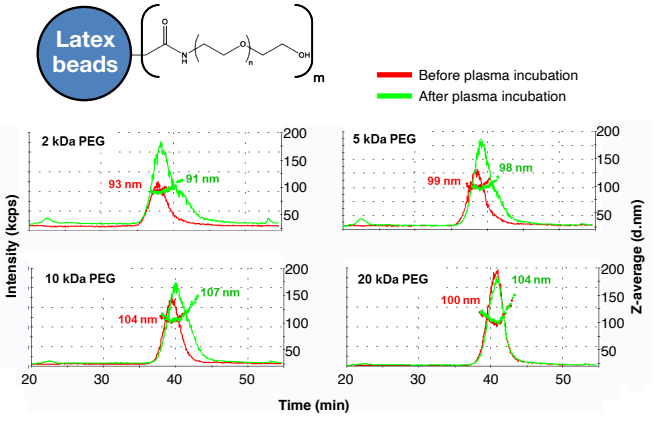
Polymer-Bound Drug Quantitation

Prodrug	CHN elemental analysis	CHNS elemental analysis	Hydrolysis	SEC-MALS/RI
Polymer Backbone (Drug, Drug, Drug, Drug)	6.5 ± 0.9%	6.6 ± 0.4%	6.7 ± 0.1% 7.4 ± 0.1%	5.7 ± 3.8%

Several orthogonal techniques to quantitate the percent of drug in a polymeric prodrug formulation. In this example, drug content was determined by elemental analysis (nitrogen and sulfur content), base hydrolysis followed by RP-HPLC separation with UV detection, and size exclusion chromatography coupled with multi-angle light scattering and refractive index detectors (SEC-MALS/RI). Adapted from Drug Deliv Transl Res, 2019, 9(6), 1057-1066.

Surface Characteristics

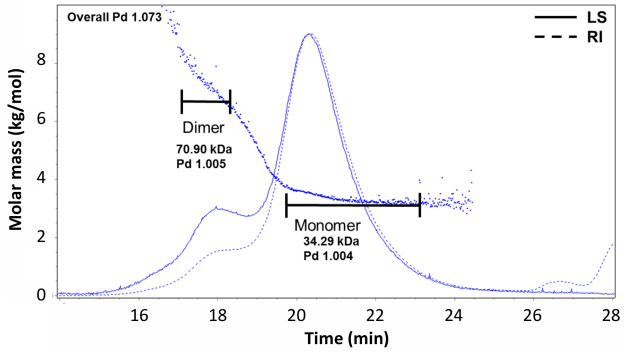
- Zeta potential
- Protein binding assessment by AF4-MALS/DLS
- Quartz crystal microbalance with dissipation monitoring (QCM-D)



Flow-mode DLS, coupled to AF4, of polystyrene beads conjugated to various lengths of PEG before and after incubation in human plasma. As PEG length increases, polydispersity decreases and there is minimal change in the size distribution pattern (red vs green trace), suggesting decreased protein binding with increased PEG length.

Purity

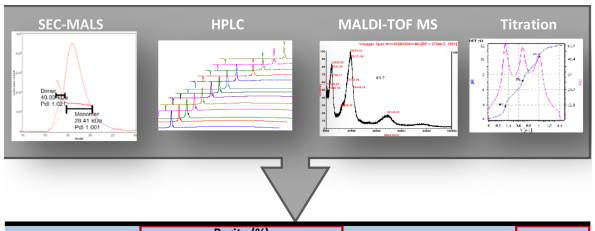
- Drug/Prodrug impurities
- Free drug/prodrug concentration
- Polymer impurities and degradation
- Polymer functionality (end-group analysis)
- Residual solvents and reagents



Purity assessment of drug-conjugated and targeted Generation 5 PAMAM dendrimers by size exclusion chromatography with MALS and refractive index detectors in-line (SEC-MALS-RI). The molecular mass distribution and polydispersity for the monomer and dimer are based on the bracketed molar mass distribution.

Batch-to-Batch Consistency

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

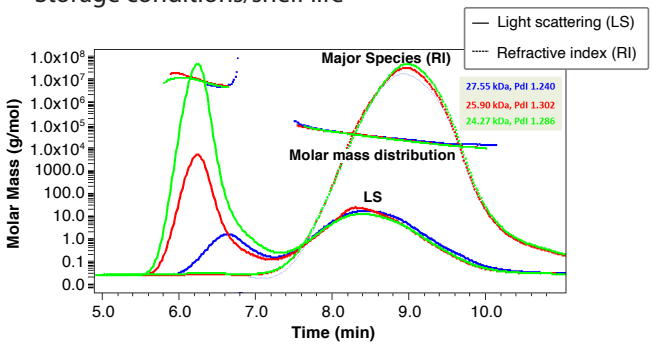


Batch No.	Vol-Peak, nm	Purity (%) G5	G4	Dimer	MW, kDa*	Zeta Potential, mV	# of NH_2
1	5.4	85.4	4.9	7.9	29.1	41.9	95
2	5.3	83.1	5.1	10.0	27.9	46.8	109
3	5.4	82.9	5.3	9.8	26.9	49.6	106
4	5.4	80.8	5.1	13.3	27.8	43.2	112
5	5.2	82.5	3.9	12.1	26.5	43.2	112
6	5.4	81.1	4.0	14.1	27.0	50.3	107
7	5.5	80.7	3.4	14.5	27.0	41.5	106
8	5.5	81.2	3.9	12.3	28.4	51.3	118
9	5.4	79.1	3.9	13.1	26.4	47.0	104
10	5.5	82.0	4.0	13.3	26.8	41.9	106
11	5.7	79.4	4.2	15.1	26.7	38.7	98
12	5.5	87.4	3.0	8.8	27.0	51.5	89
13	5.4	86.6	2.9	9.6	27.3	37.7	NA

Batch-to-batch consistency for G5 amine-terminated dendrimers was assessed by several techniques. In this case, HPLC and titration were the relevant characterization methods.

Starting Material Characterization

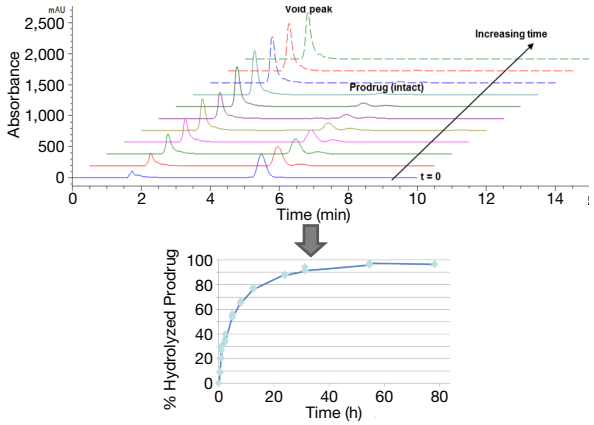
- Drug/prodrug identity (structure)
- Drug/prodrug purity (degradation products)
- Polymer composition (molecular mass and polydispersity)
- Polymer purity and functionality
- Storage conditions/shelf life



Polymer composition and molar mass distribution of mPEG-PLGA were determined by thermogravimetric analysis and SEC-MALS-RI (above), respectively.

Stability

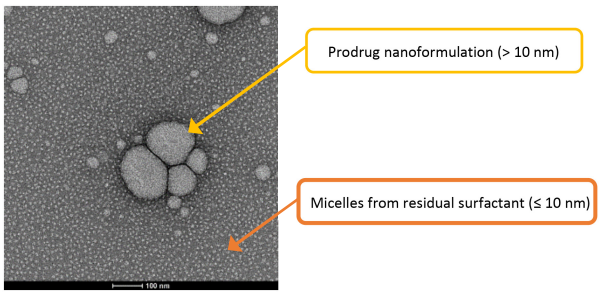
- Size/Size distribution; aggregation
- Drug leakage and degradation
- Polymer degradation
- Drug/prodrug release in plasma
- Solvent, thermal, pH, photo, freeze-thaw, lyophilization, centrifugation, filtration
- Storage conditions/shelf-Life



Stability assessment of prodrug micelles in the presence of human plasma. The hydrolysis of the intact prodrug was measured by RP-HPLC and used to construct a hydrolysis versus time graph, which can be used to determine t½.

Morphology

- Transmission electron microscopy (TEM)



Representative TEM image of PEGylated oil-filled prodrug nanocapsules. In addition to determining the size distribution, TEM can be used to evaluate morphology and purity. Here, the presence of residual surfactant forming smaller micelles are observed in the background.

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
Drug Deliv Transl Res, 2019, 9(6), 1057-1066. PMID: 31119521
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
Polymer nanoparticles: A Guide for Design, Preparation and Development as Nanomedicines, 2016, 187-203. ISBN: 978-3-319-41421-8
Anal Bioanal Chem, 2015, 407(29), 8661-8672. PMID: 26449845

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

Email us at ncl@mail.nih.gov

DOI: textbox placeholder for information.