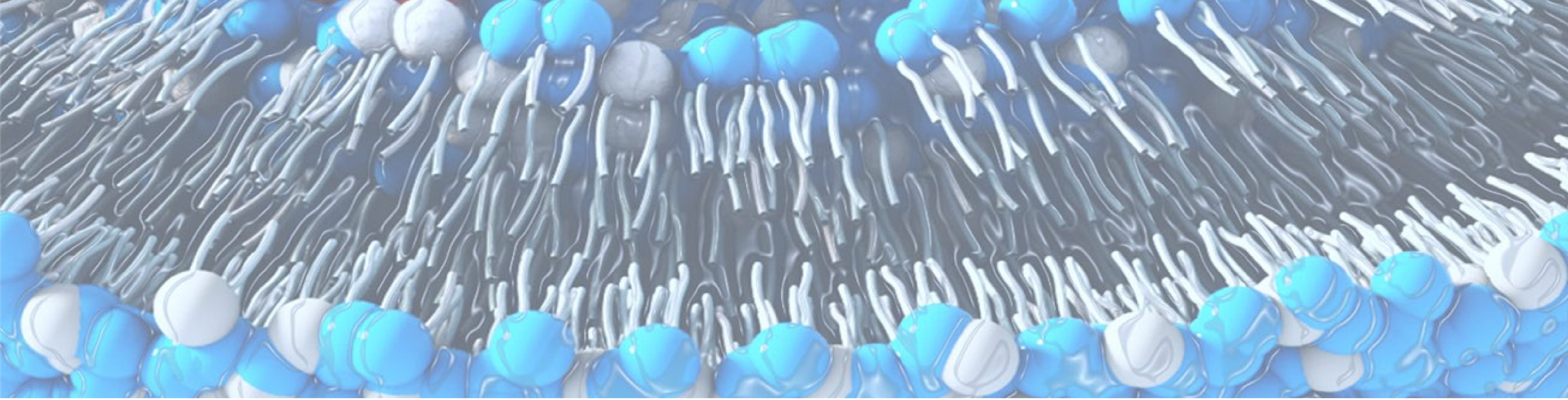


Continuous Manufacturing Platform for Lipid and Polymer-based Nanoparticle Therapeutics

Presenter: Antonio Costa, Ph.D.
Diane Burgess, Ph.D.

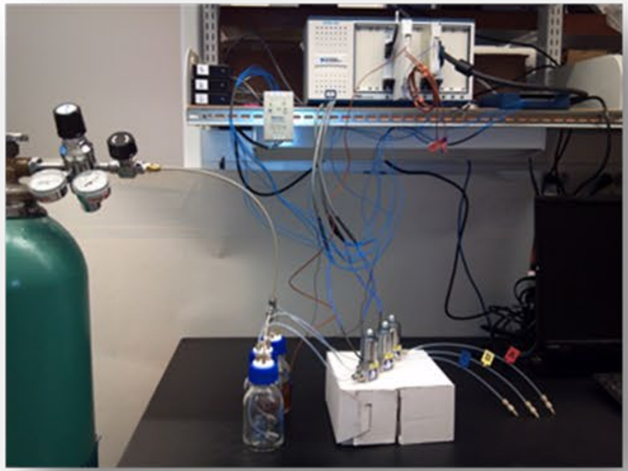
Affiliation: University of Connecticut, Storrs, CT

10/11/2023

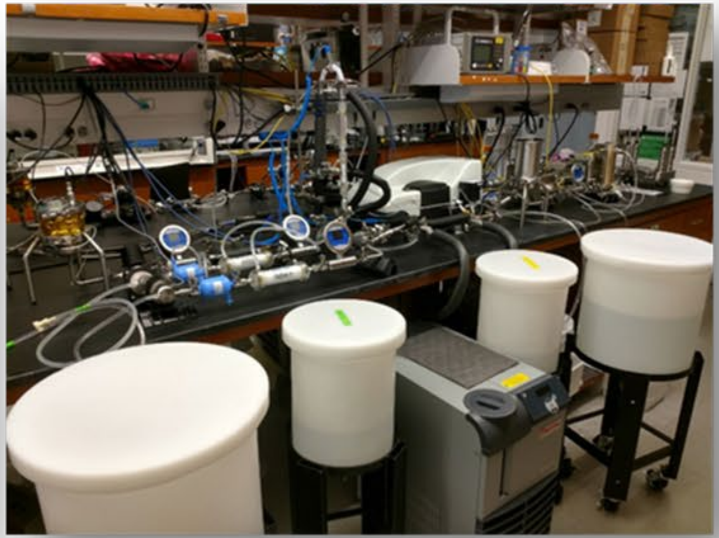
Disclaimer: This presentation reflects the views of the presenter and should not be construed to represent FDA's views or policies. Dr. Diane Burgess and Dr. Antonio Costa may have a potential conflict of interest concerning DIANT Pharma Inc., as some of the technology used in this work is licensed to DIANT Pharma Inc..

From laboratory to industrial technology

2014



2018

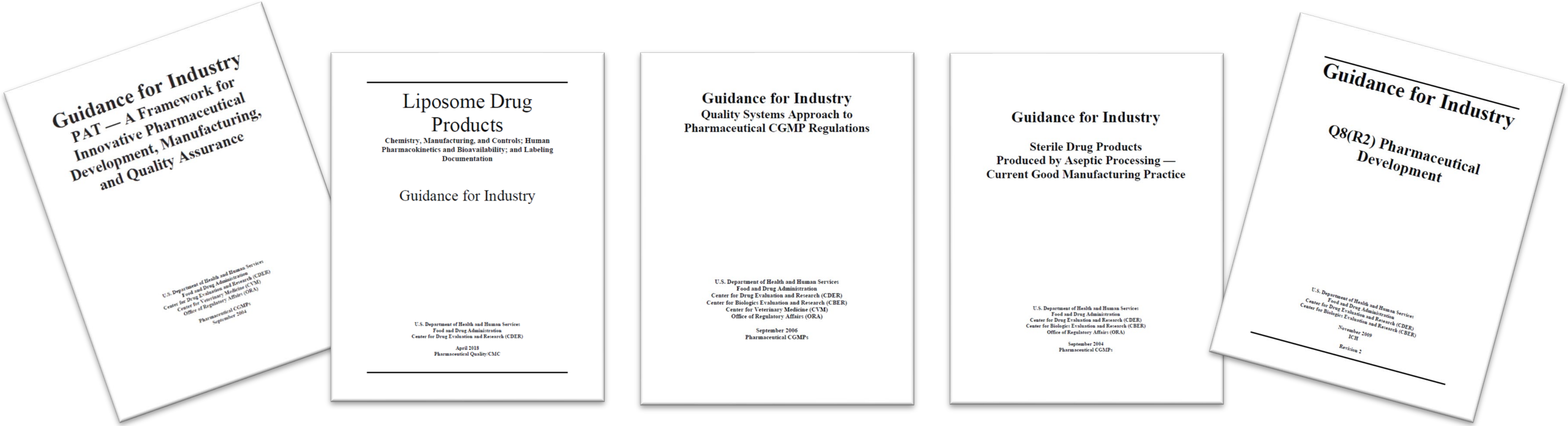


2022

Modernizing Pharma Manufacturing

"One of today's most important tools for modernizing the pharmaceutical industry is a process known as continuous manufacturing…"

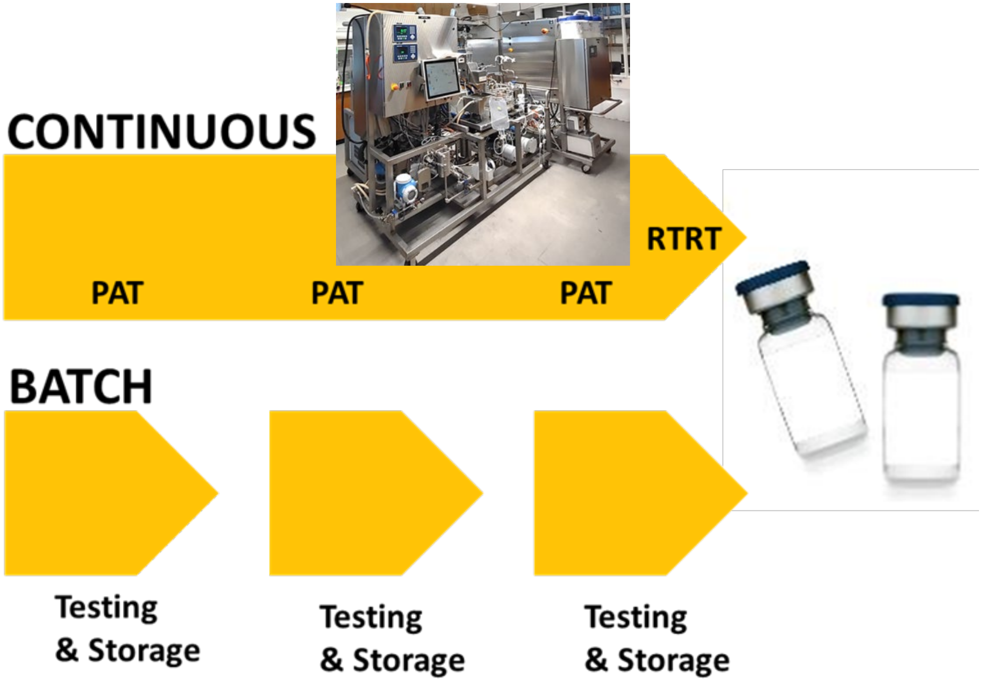
~Joint Statement by former FDA Commissioner Scott Gottlieb and FDA Director Janet Woodcock, 2019



"Allows FDA to issue grants to study continuous manufacturing — a technologically advanced and automated manufacturing method."

~The 21st Century Cures Act, 2019

Batch vs. Continuous



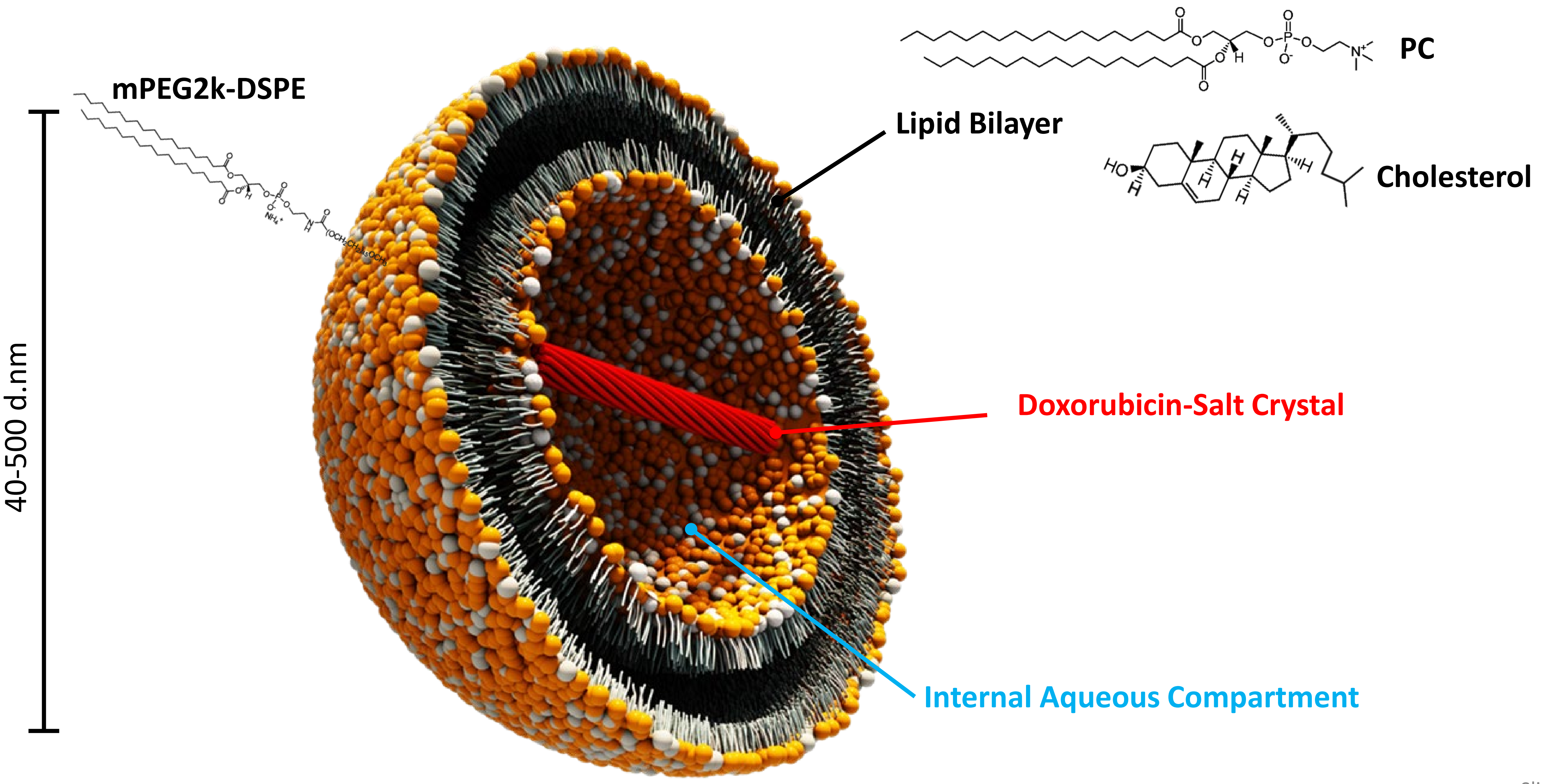
"…quality cannot be tested into products; it should be built-in or should ***be*** *by design"*

FDA Guidance on PAT Framework

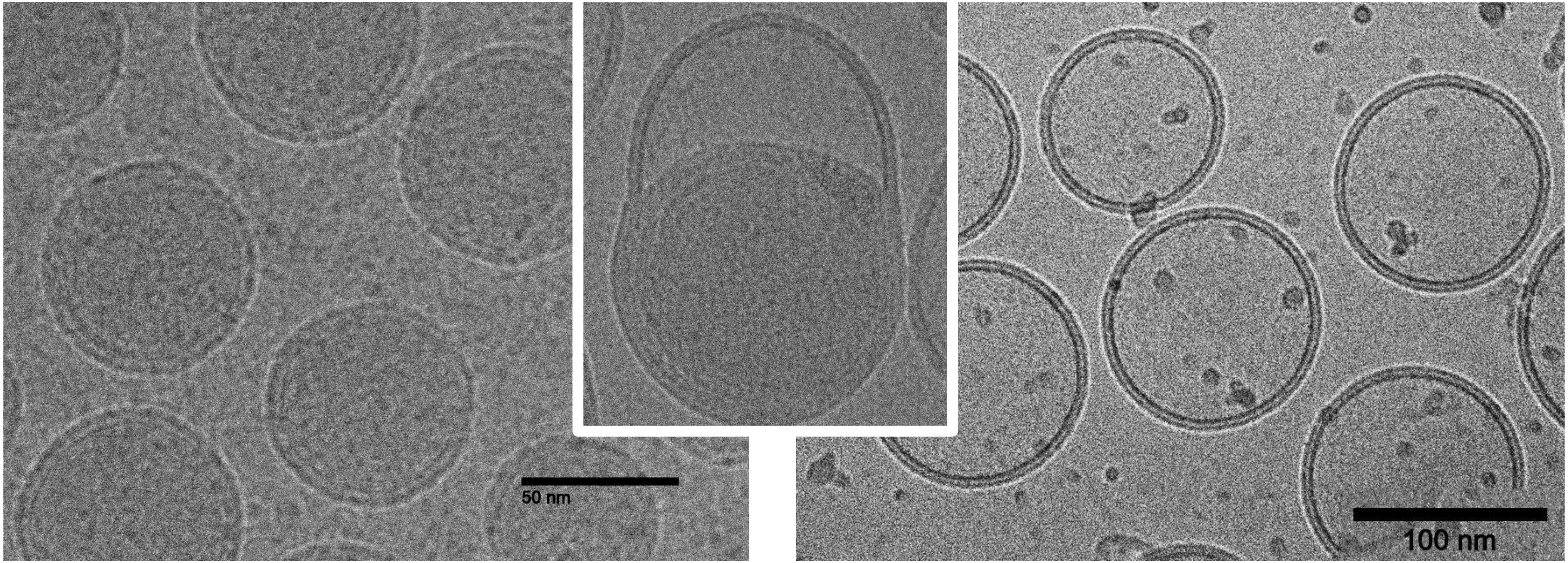
Continuous Manufacturing Benefits

- **Higher Quality Products**
- **Faster to Market**
- **Scalable**
- **Process Analytical Technology**
  - Fine-Control of Quality Attributes
- **Reduce Product Waste**
  - Divert Process if Error Occurs
- **Reduce System Footprint**
  - Portable System
- **Reduce Human Error**
  - Reduction in Open Transfers
- **Supports End-to-End Manufacturing**

Liposomal Nanoparticle Properties and Structure

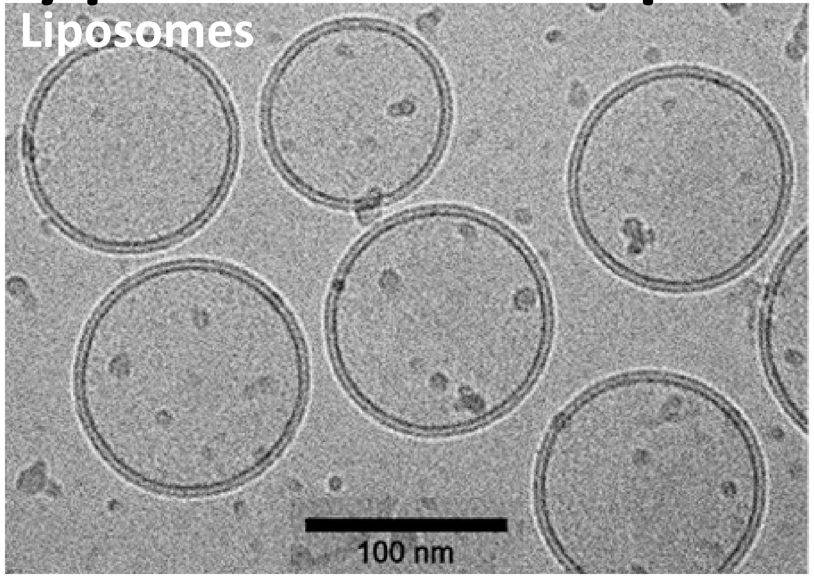


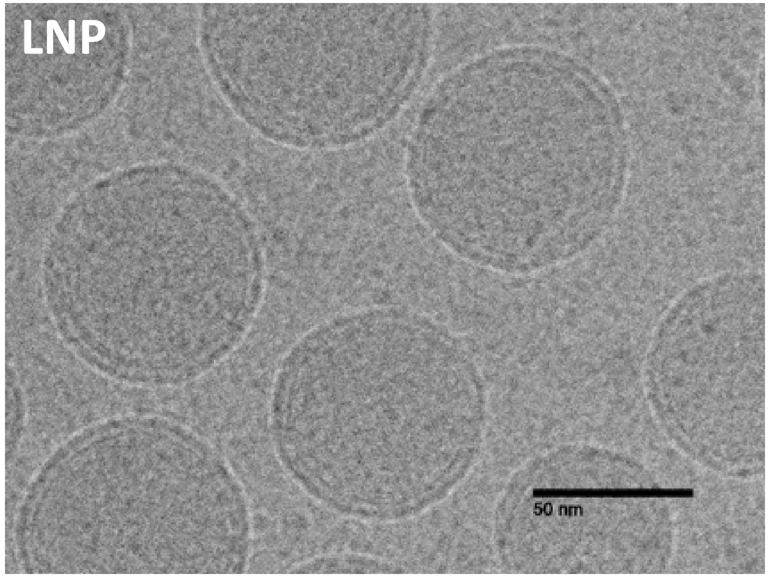
LNP vs Liposome Structure

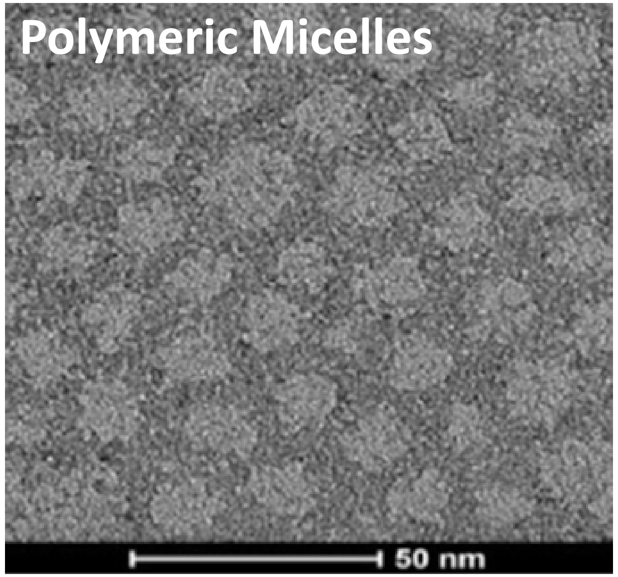


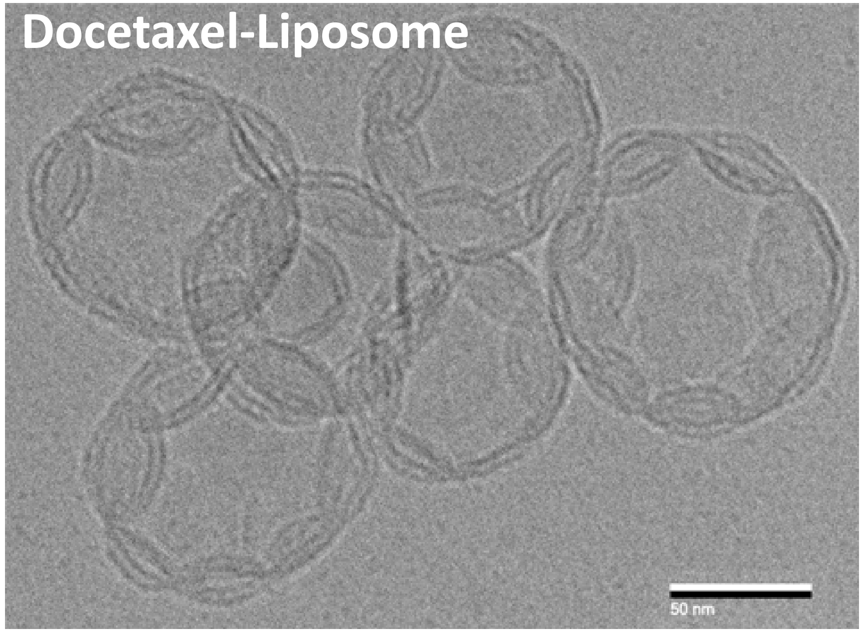
Lipid Nanoparticles	*Liposomes*
1. PC Lipid	1. PC Lipid
2. Cholesterol	2. Cholesterol
3. Pegylated Lipid	3. Pegylated Lipid
4. Ionizable Lipid	
5. Nucleic Acid	

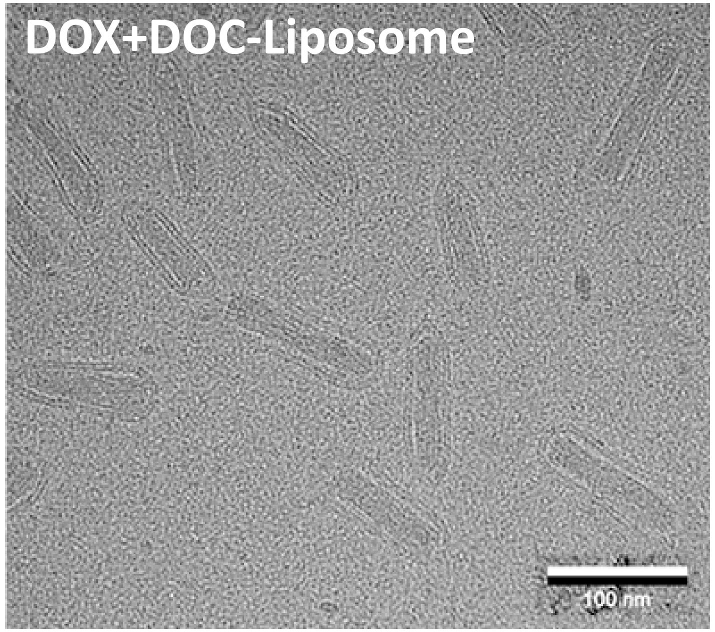
Types of Nanoparticles

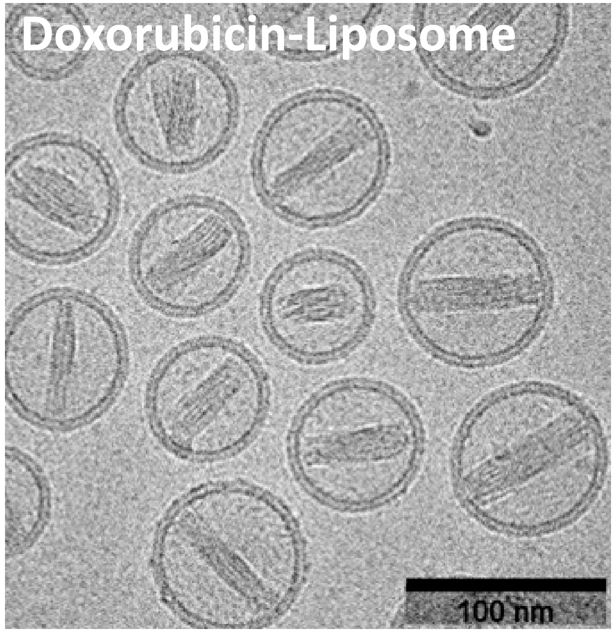




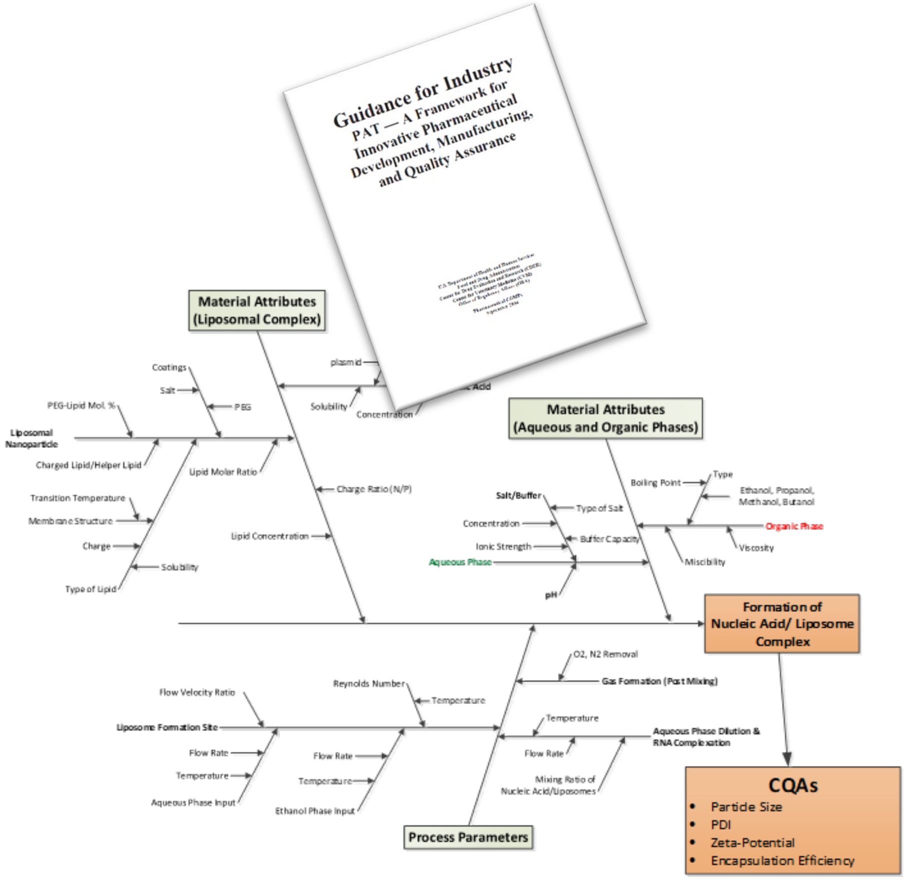








Quality by Design - A risk-based approach to the problem



Example Ishikawa Diagram to relate material attributes and process parameters to cQAs.

Material Attributes

- Lipid Concentration
- Lipid Molar Ratios
- Lipid Purity
- Lipid pKa
- Lipid Headgroup
- Lipid Ionizable Species
- Aqueous Phase
  - Salt Additions
  - Organic Phase Additions

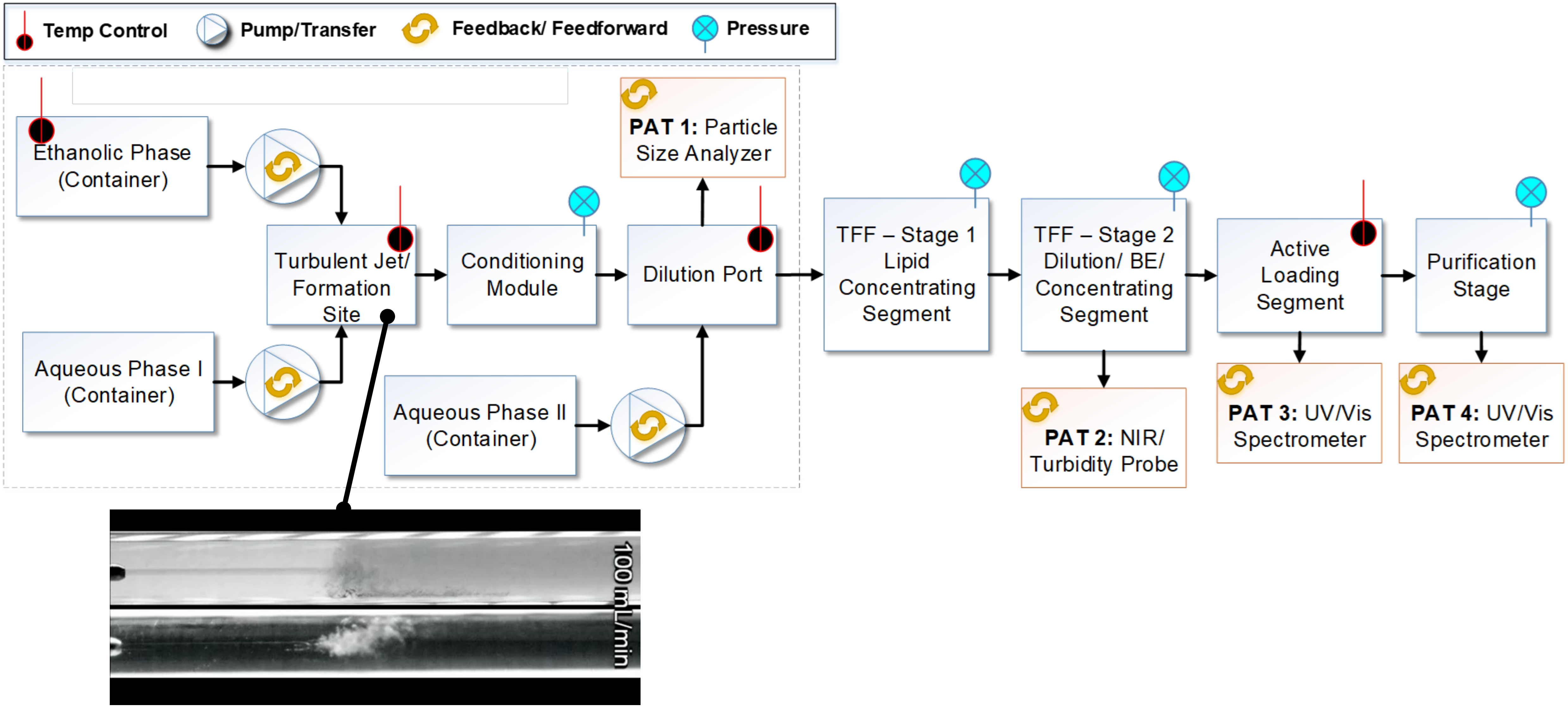
Process Parameters

- Aqueous Flow Rates
- Ethanol Flow Rates
- Reynolds Number of Mixing
- Aqueous Phase Temperature
- Ethanolic Phase Temperature
- pH of Aqueous Phase

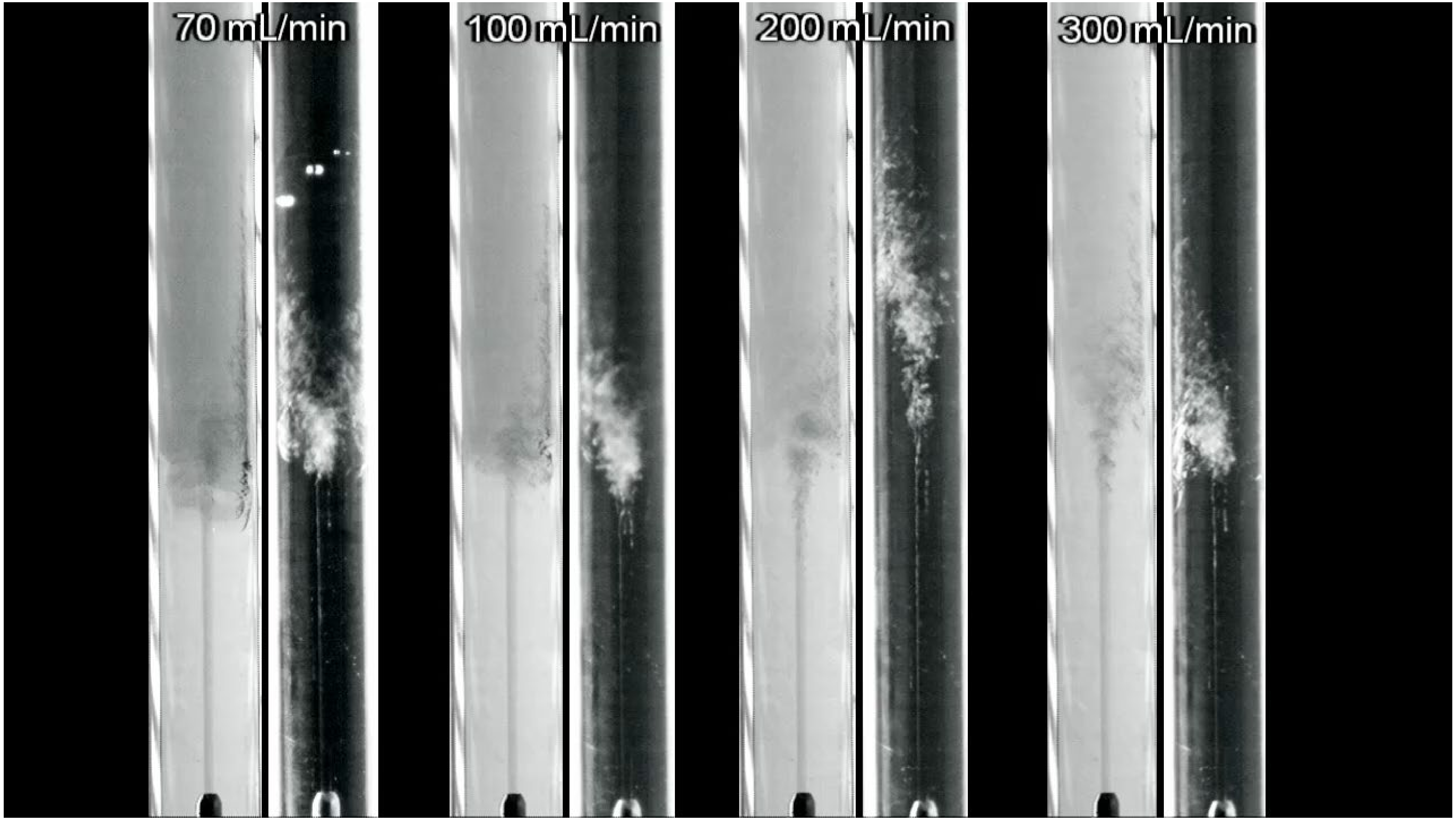
Quality Attributes

- Particle Size
- Polydispersity Index (PDI)
- Zeta-Potential and Deviation
- Encapsulation Efficiency
- Total Drug
- Drug Loading
- Drug Crystal Structure
- Residual Solvent

Nanoparticle Process Flow Chart

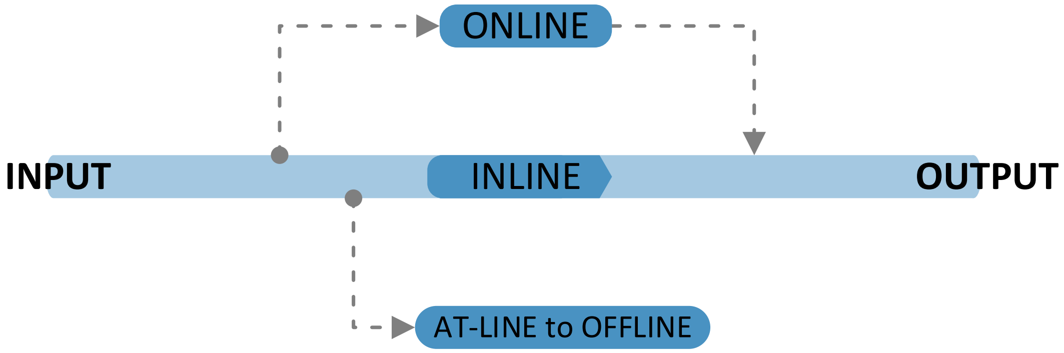


High-Speed Camera: Jet Formation



Entire 30 second video takes place in less than 1 second…

Process Analytical Technology

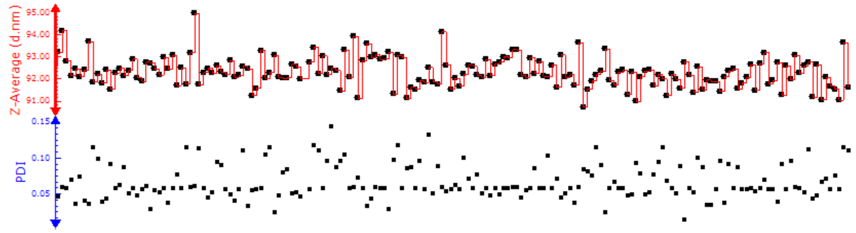


PAT Tools: Process analyzer or one or more “soft sensors” with predictive algorithms (multivariate) to determine critical attributes.

DLS

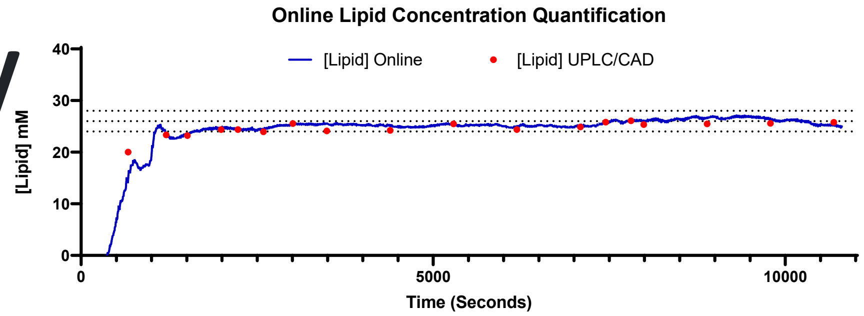
Z-Average

PDI



Turbidity

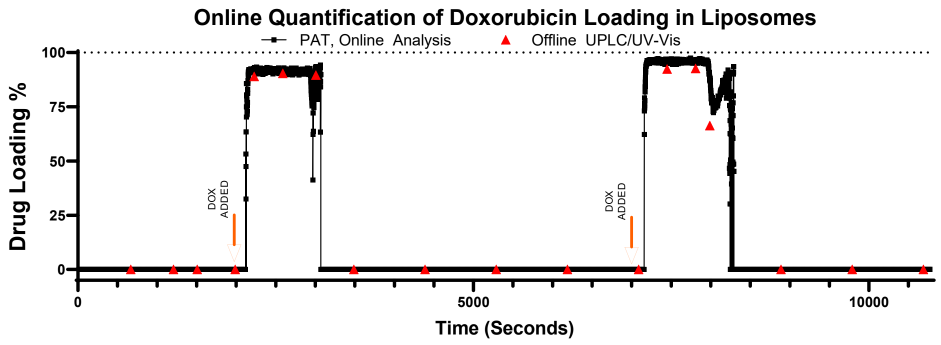
[Particle]



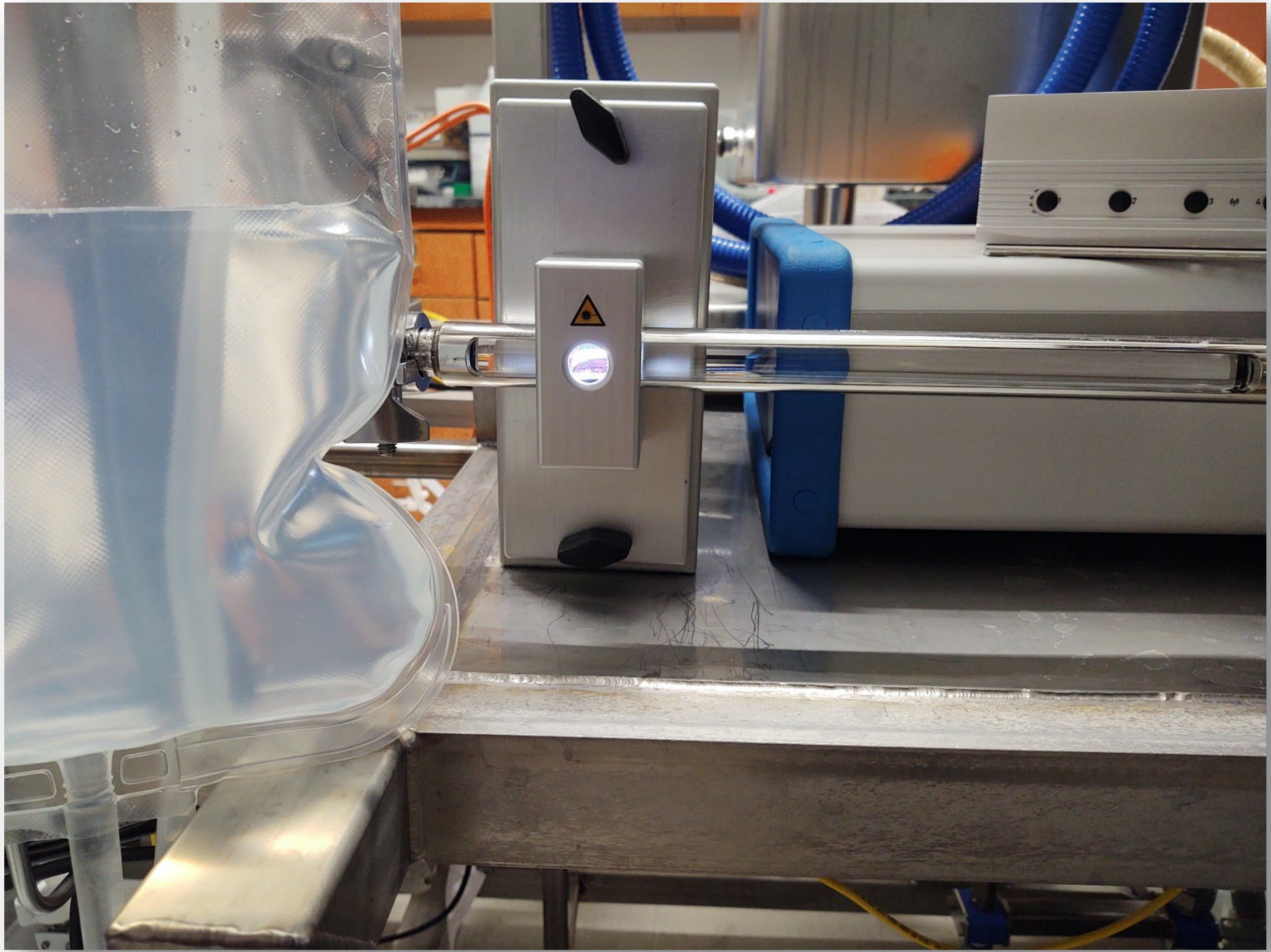
UV/Vis

[Drug]

%E



PAT Integration: Particle Size Setup



INPROCESS | LSP

NanoFlowSizer

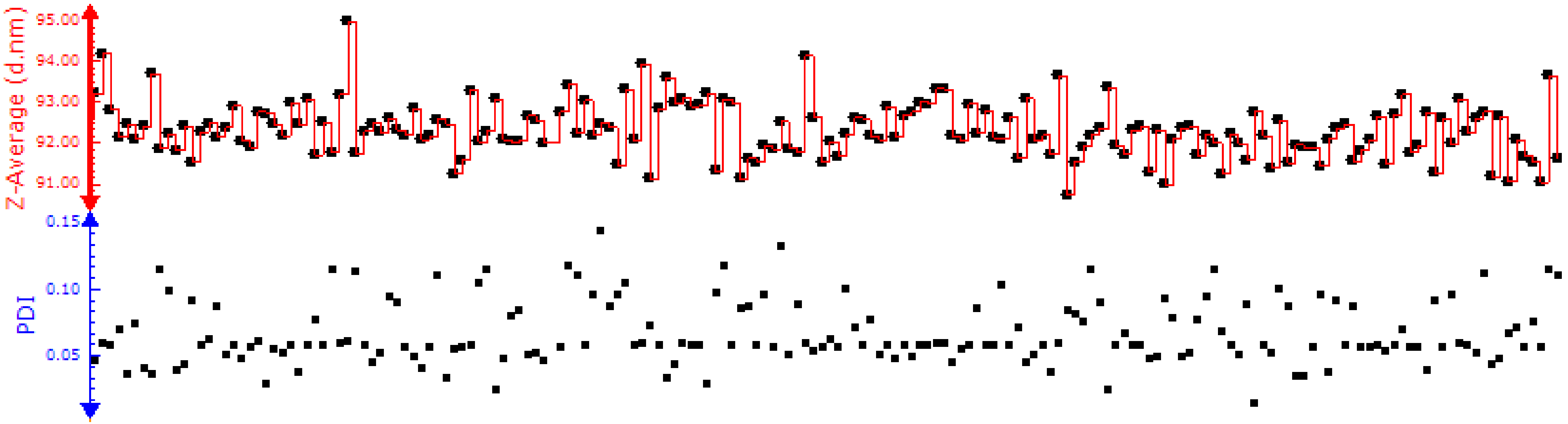
Spatially Resolved-DLS

Mode: Online
Measurement Interval: <6s

Benefits

- Rapid, online analysis
- Measurements at high flow rates
- Low and high concentrations acceptable
- Compliant Software package

Monitor and Control of Particle Size



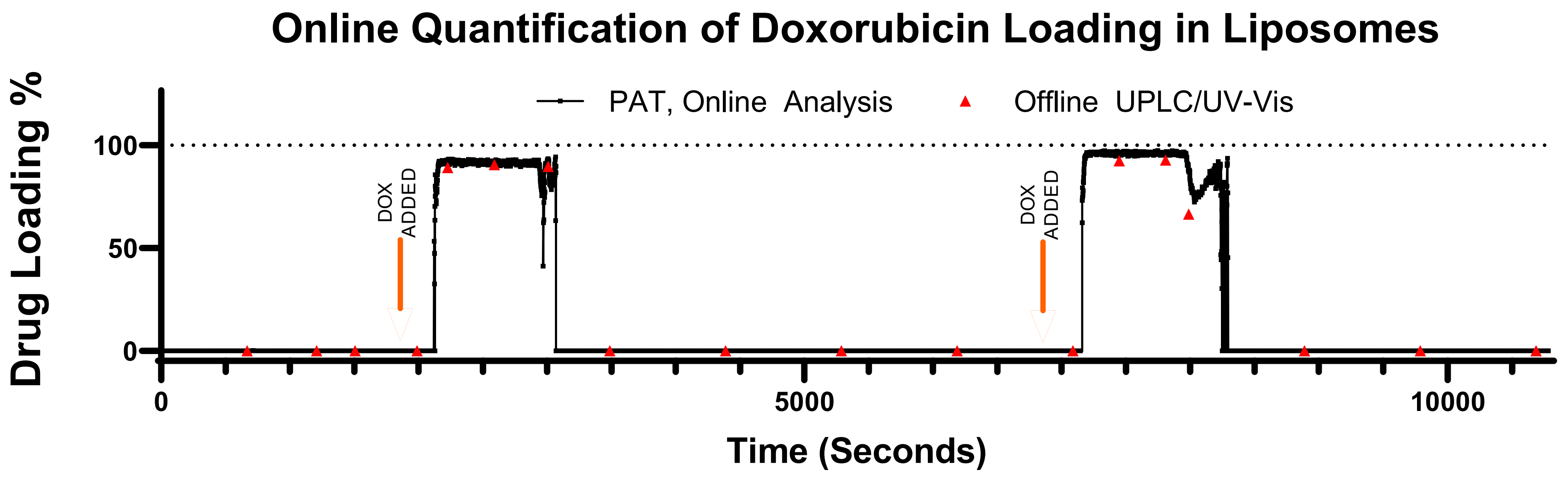
Measured Attributes

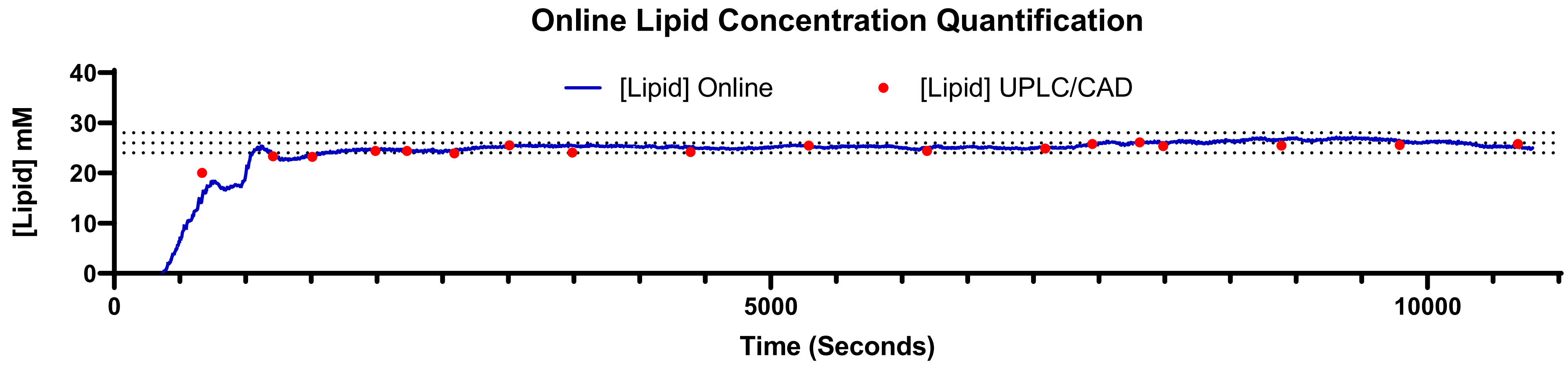
1. Z-Average (d.nm)
2. CumulantPDI
3. Shear Rate
4. Temperature

Relate intermediate liposomes at formation site to liposomes as the "end-product".

Predictive behavior of intermediates to "end-product"?

Online Doxorubicin and Lipid Concentration Analysis





DOE Example: Material Attributes

Multi-factorial Design (5x3x2x3x3 = 270 runs)

Lipid Formulation: (Main Lipid Type):Chol:DSPG

DSPG was fixed at 5% molar ratio.

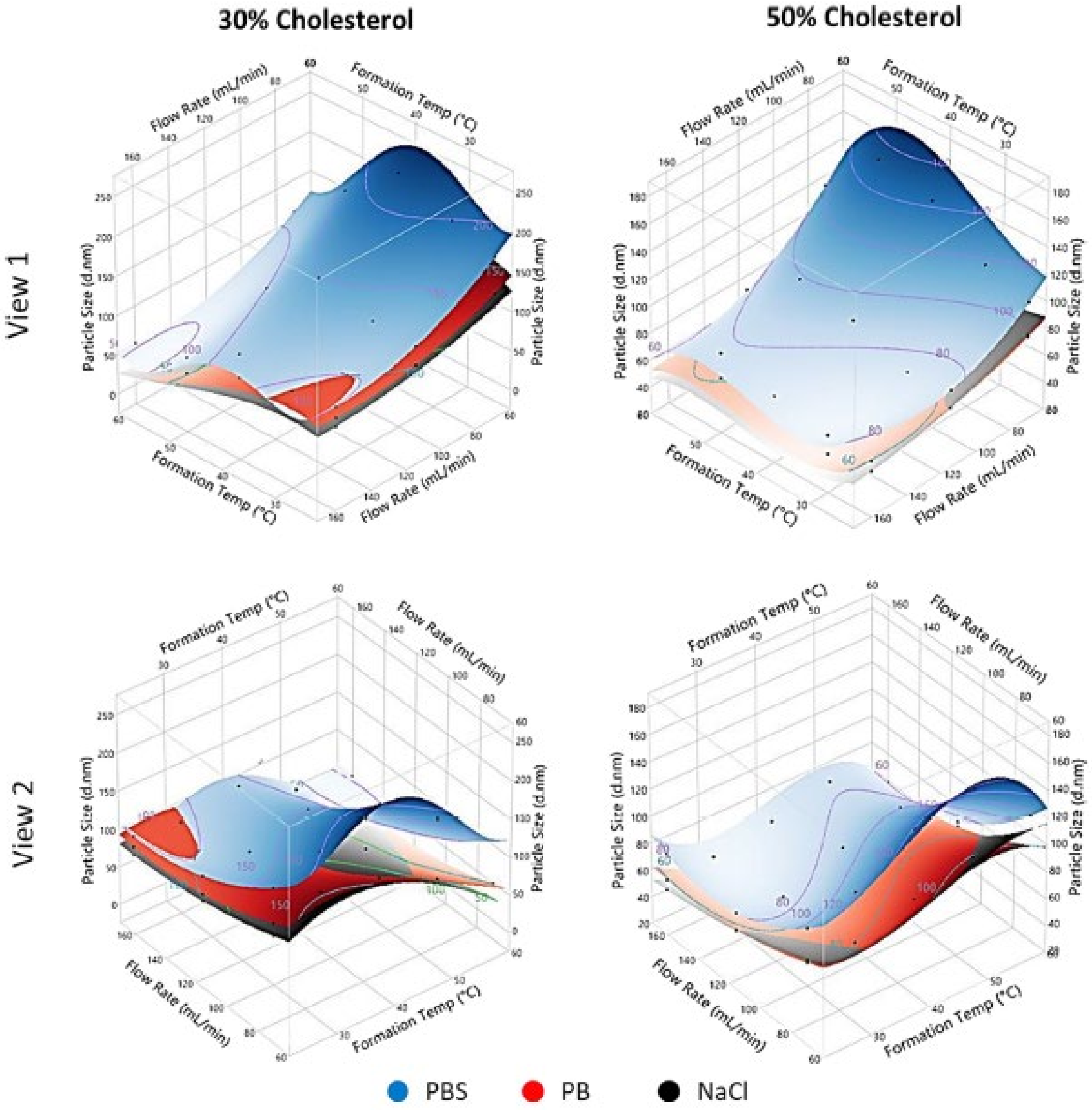
Factors

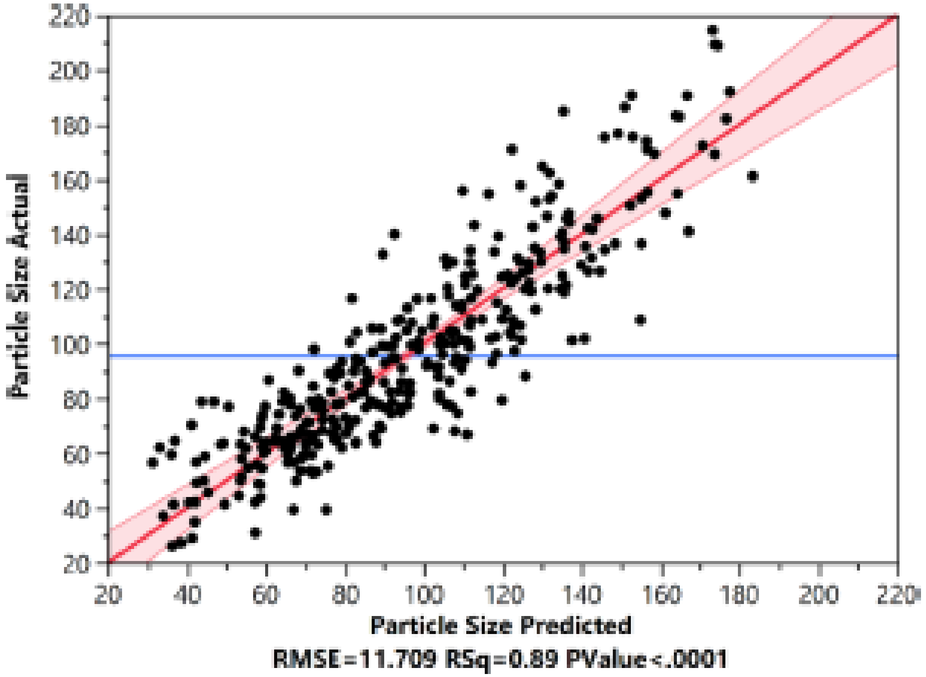
- Post Formation Temperature (20-60°C)
- Aqueous Phase Flow Rate (70-160 mL/min)
- Cholesterol Percentage (30-50%)
- Main Lipid Type (**DMPC** vs **DPPC** vs **DSPC**)
- Aqueous Phase Salt (**PBS**, **PB** or **NaCl**)
  - pH set to 7.45 for PBS and PB.

Responses

- Z-Average Particle Size
- PDI (Polydispersity Index)
- Zeta-Potential

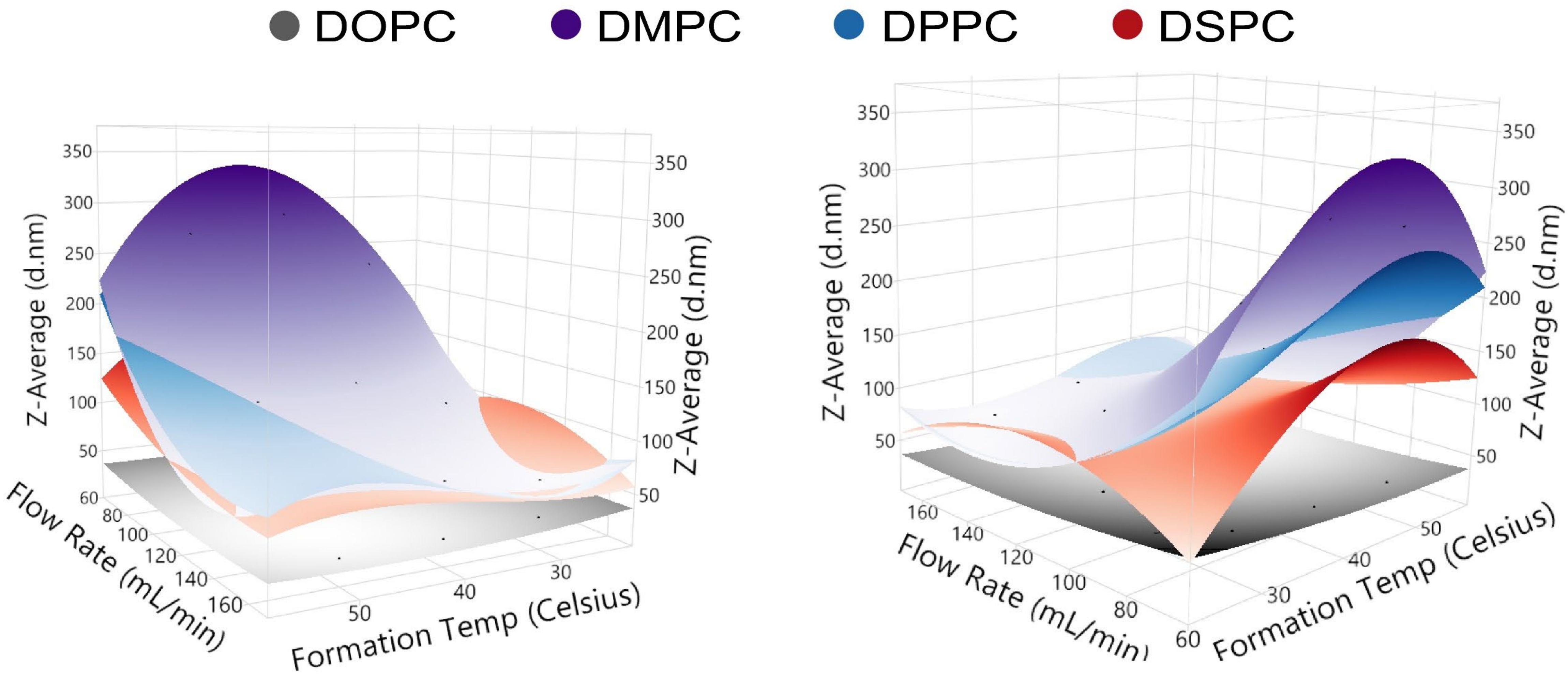
HSPC-based liposomes





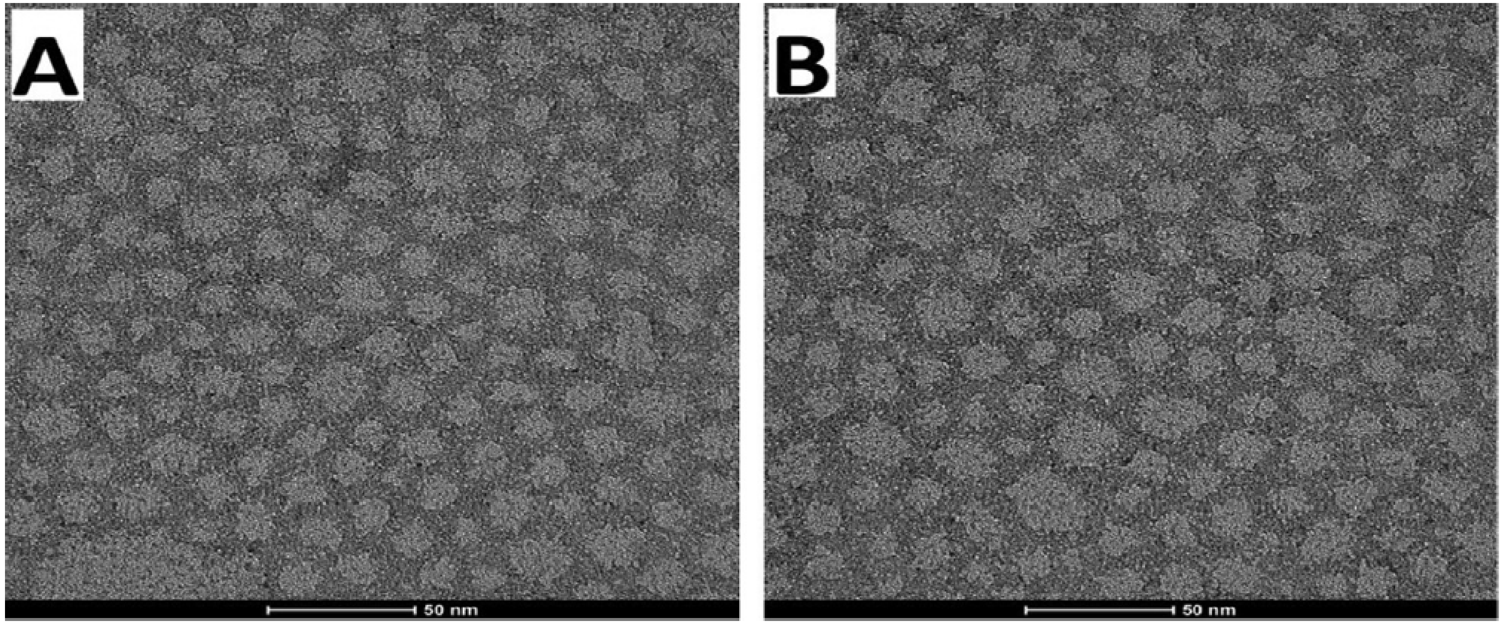
Gowtham Yenduri, Antonio P. Costa, Xiaoming Xu, Diane J. Burgess, Impact of critical process parameters and critical material attributes on the critical quality attributes of liposomal formulations prepared using continuous processing, International Journal of Pharmaceutics, Volume 619,2022, 121700,ISSN 0378-5173,

Type of Lipid on Size

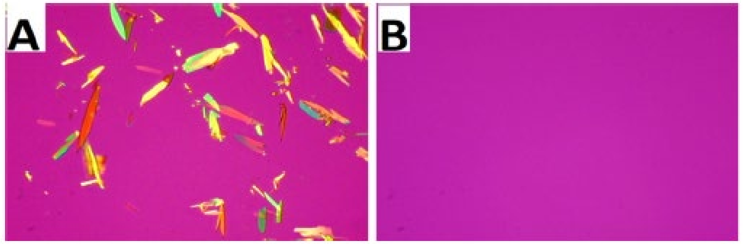


View 1 View 2

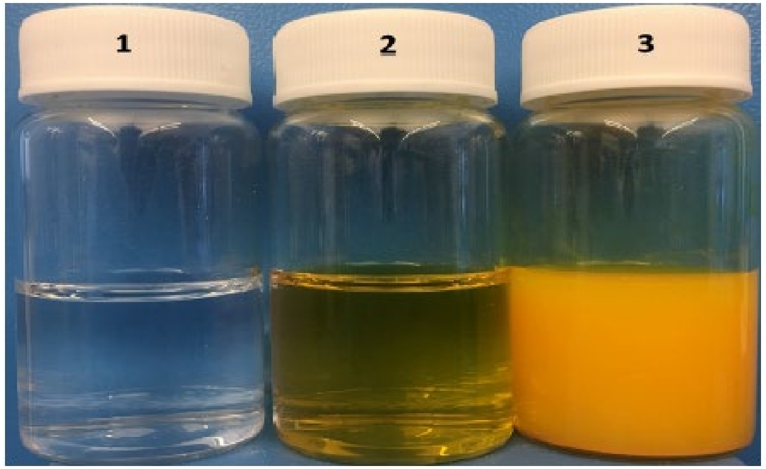
Formulation and Characterization of Polymeric Micelles



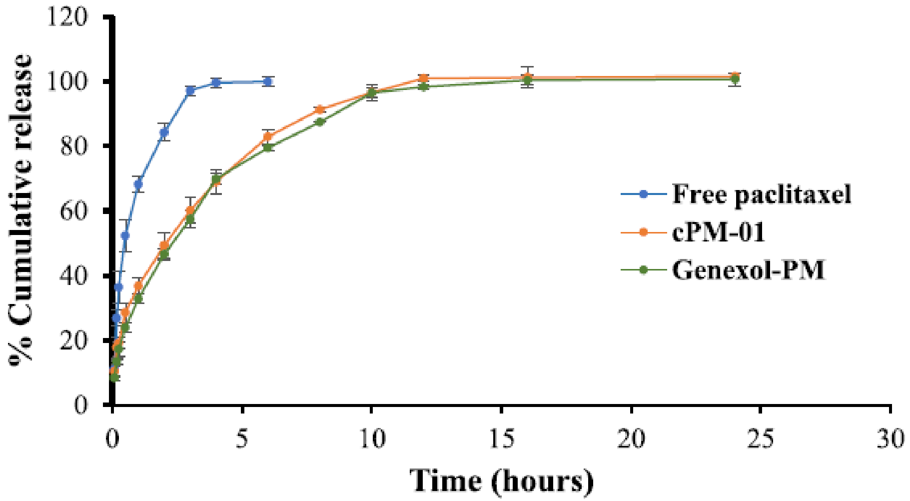
Transmission electron microscopy images of: (A) blank; and (B) curcumin-loaded micelles.



Polarized light microscopy images of: (A) free curcumin (5% ethanol in water); and (B) curcumin-loaded polymeric micelles (5% ethanol in water).



Appearance of: (1) blank polymeric micelles, transparent; (2) curcumin-loaded polymeric micelles, yellow transparent; and (3) free curcumin in water, yellow opaque.



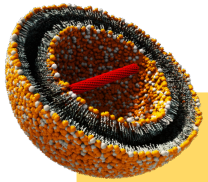
Formulation and characterization of curcumin loaded polymeric micelles produced via continuous processing, International Journal of Pharmaceutics 583 (2020) 119340

Case Study: Liposomal Doxorubicin

Performed at

URI Pharmaceutical Development Institute Training Center

Liposomal Doxorubicin Specification



Parameters	Brand Name/ Batch Process	Continuous Processing
Lipid composition	HSPC/Chol/mPEG2000-DSPE (56.3:38.4:5.3 mol%)	
[Lipid]	20 mM	
[Doxorubicin-HCl]	2 mg/mL	
Drug Encapsulation	>90% Leaflet 99% Tested	>95% No Purification >99% Post Purification
Loading Battery	Ammonium sulfate 250mM	
External Buffer	10mM histidine, pH 6.5 + 10% Sucrose	
Particle Size (d.nm)	80-85 nm (DLS)	

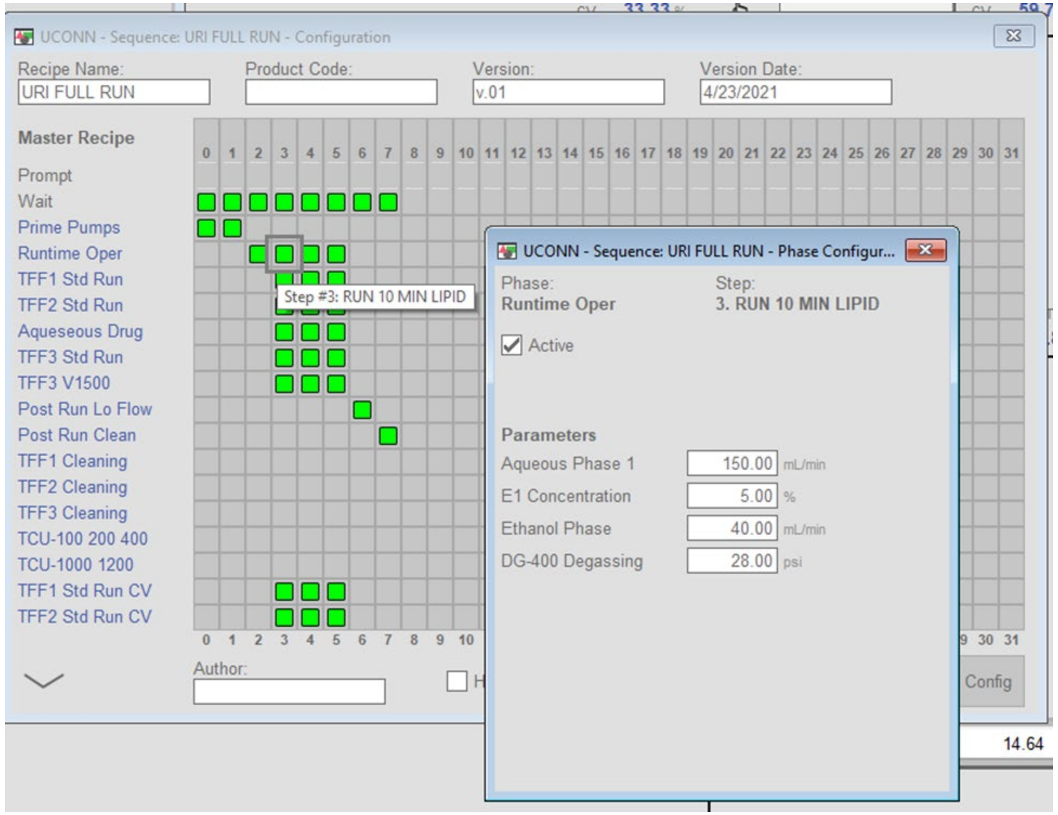
System in Clean-Room Suite

Cleanroom at URI, UConn System front and back views

Procedures and Overview

1. Gowning procedures
2. Draft batch record
3. Draft cleaning SOP
4. System SOPS
   - Run-time Recipes
5. Environmental monitoring report
6. Cleaning cycle development report
   - Executed UCONN cleaning cycle form
7. Personnel monitoring

Example Run-time Recipe

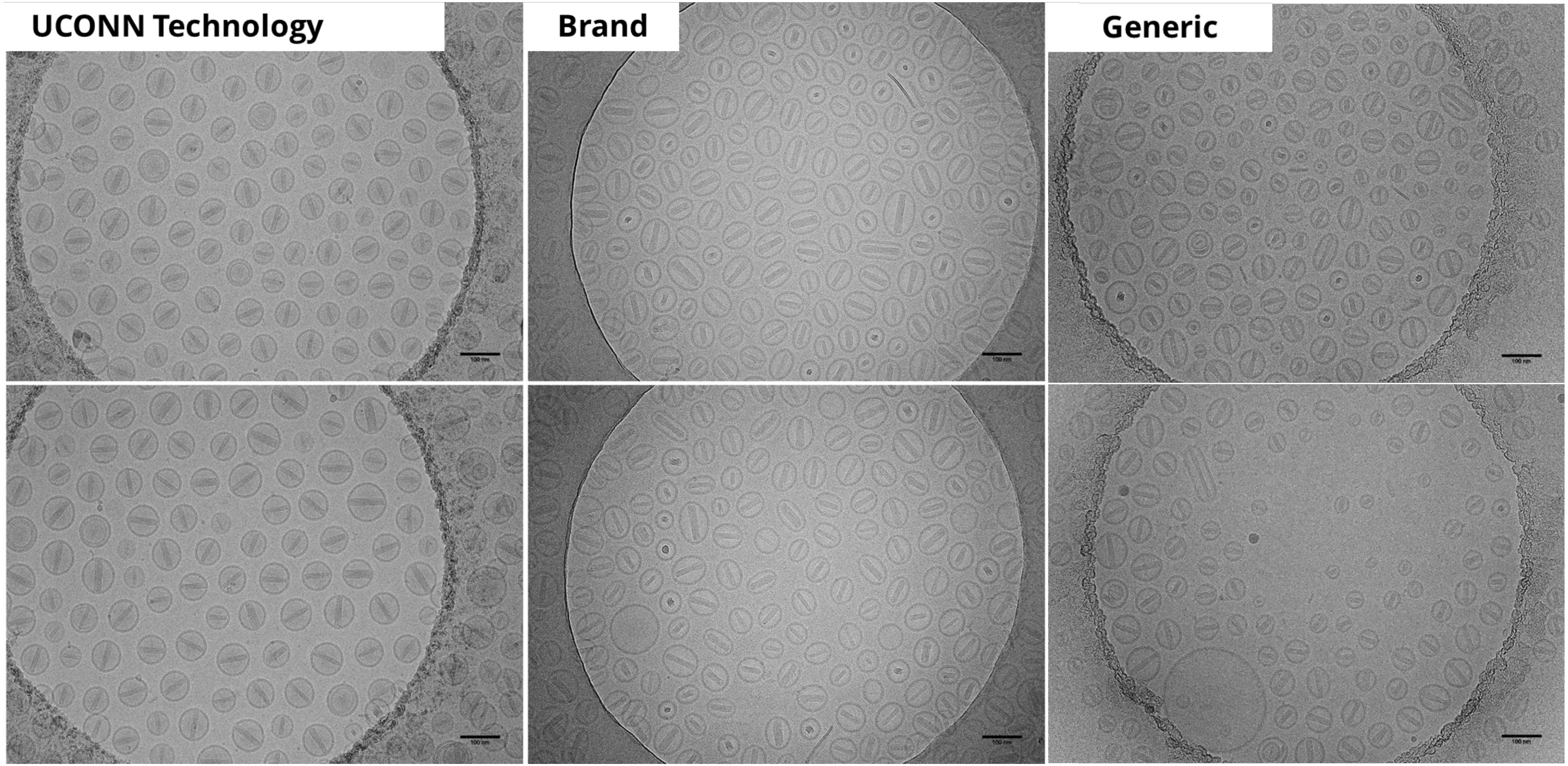


Final Particle Size Results (n=3)

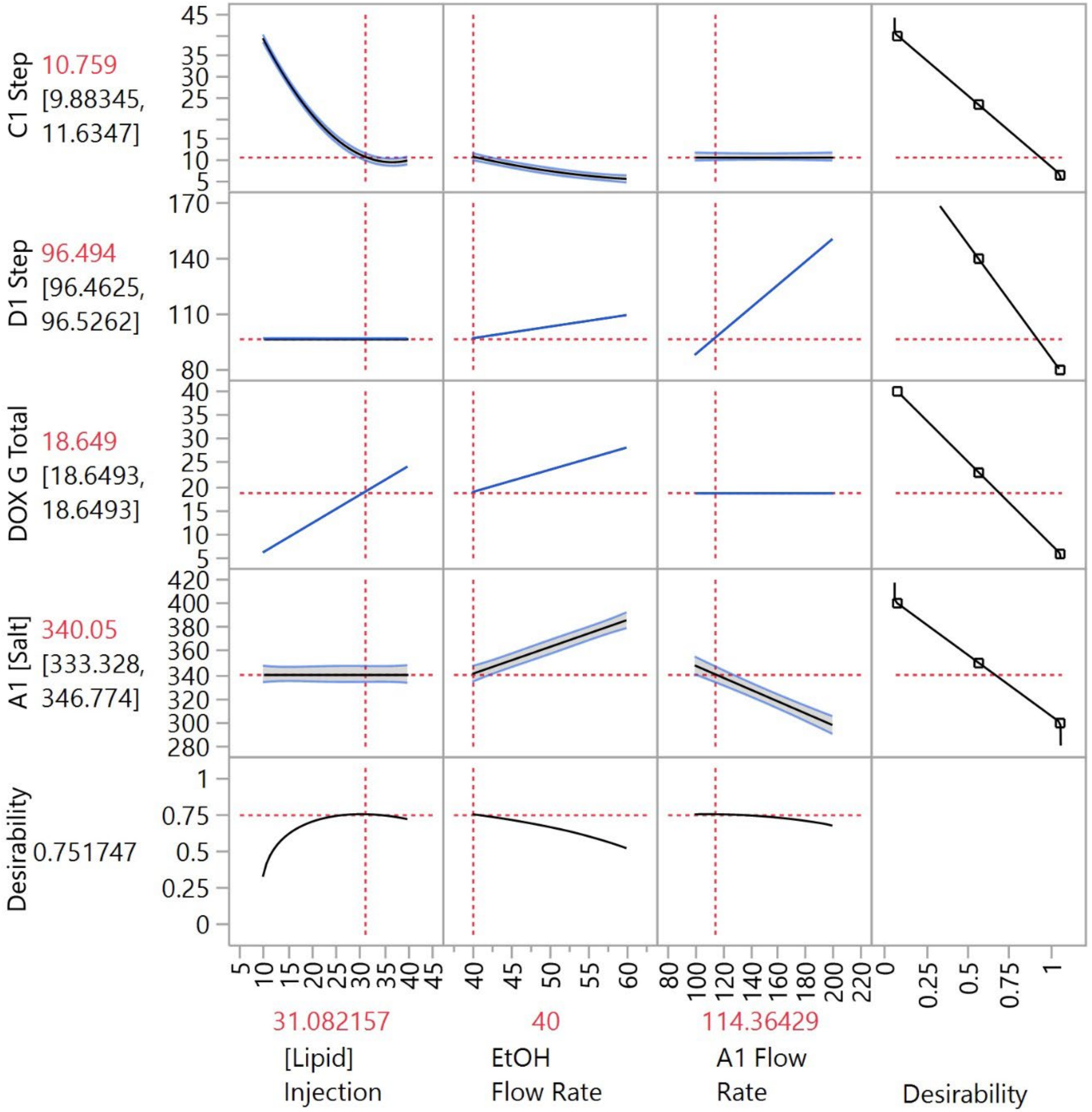
Sample Name	Z-Ave (d.nm)	PDI	EE (%)
D29t-Run 1	87.54	0.027	85%
D29t-Run 2	86.45	0.035	83%
E5t-Run 3	87.94	0.035	90%
AVERAGES:	87.3	0.032	86.0%
STDEV:	0.771	0.005	3.6%

- Three runs with cleaning in-between each run cycle.
- EE% is the drug encapsulation of doxorubicin in the liposomes.
  - Lower than expected, heating stage was lower.
- Runs were successful.
  - Low standard deviations and percent errors are reported.

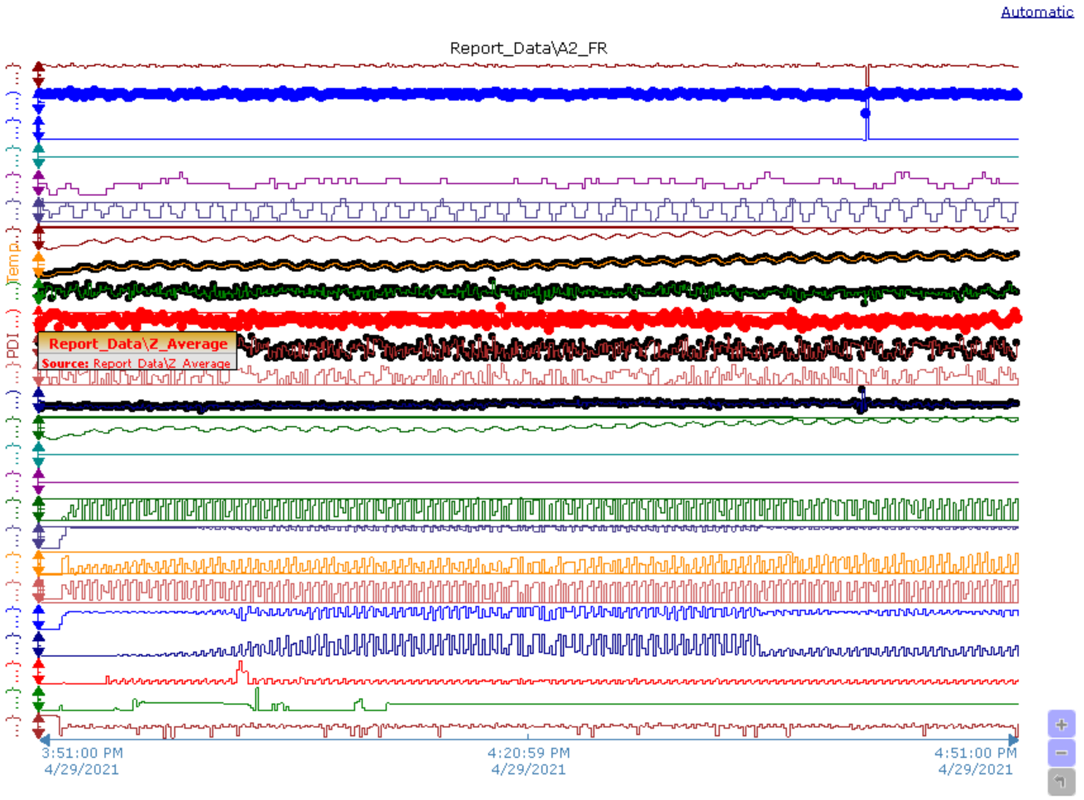
Cryo-TEM of Liposomal Doxorubicin



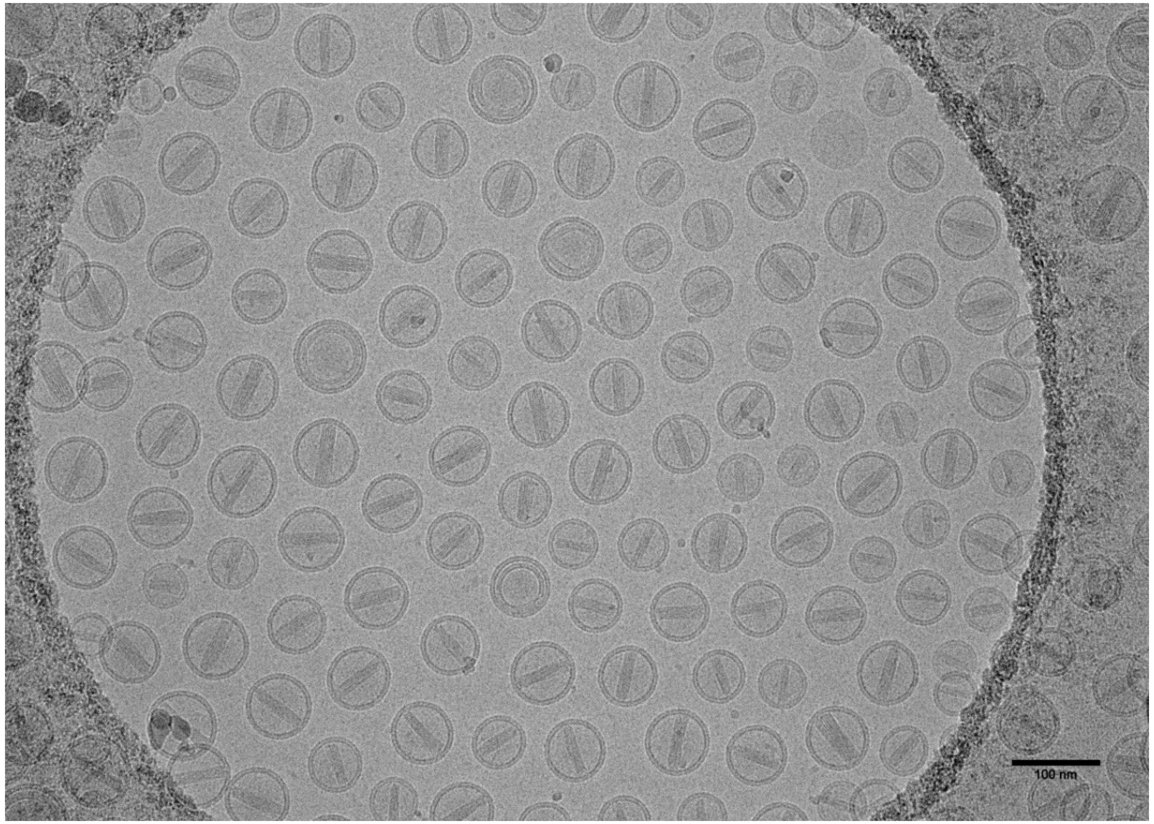
Control Strategy: towards a working Design Space



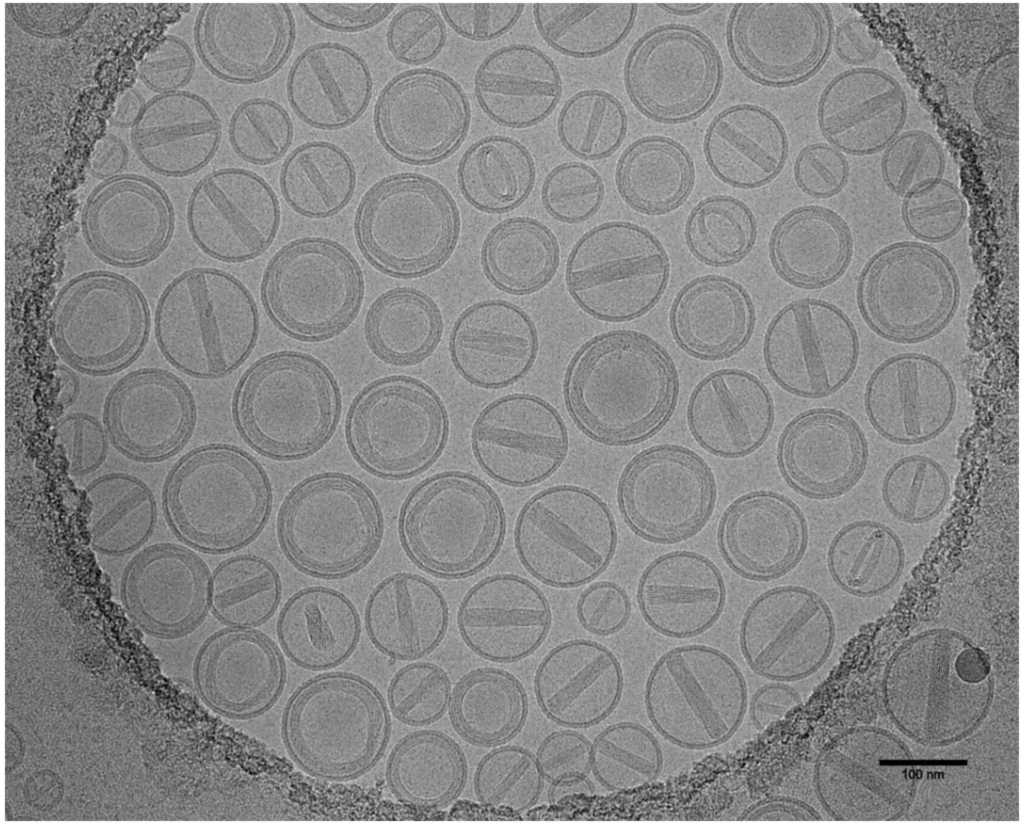
Use of Design of Experiments provides a framework to optimize conditions and run automation.



Controlled API/ Crystal Morphology (Anti-cancer)



DLS: 86.68 d.nm

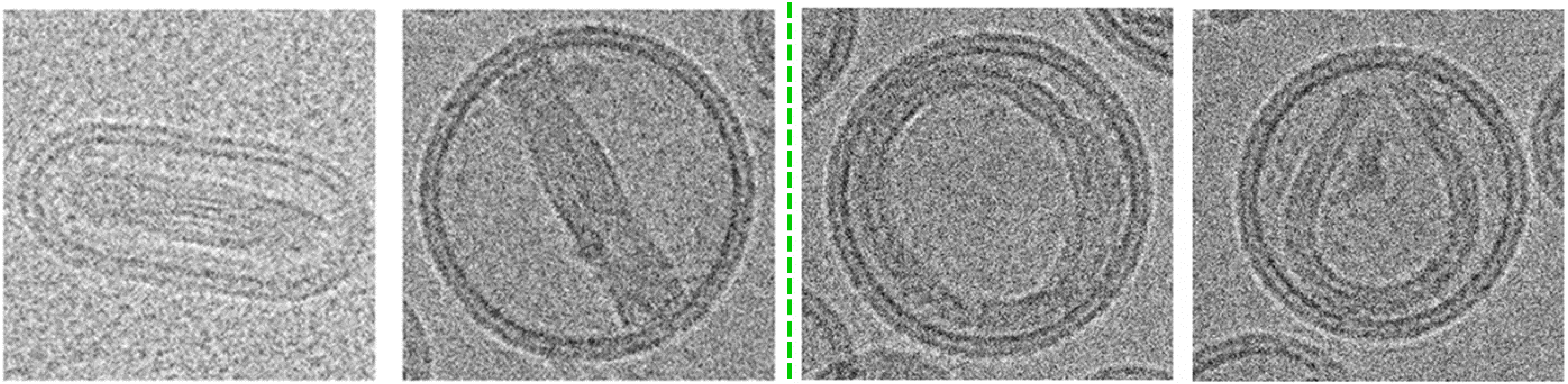


DLS: 123.33 d.nm

Linear crystal vs. spherical crystal
For small-molecule anti-cancer therapies

Structures in Liposomal Doxorubicin

Majority of Particles



Elongated, Linear Nanocrystal

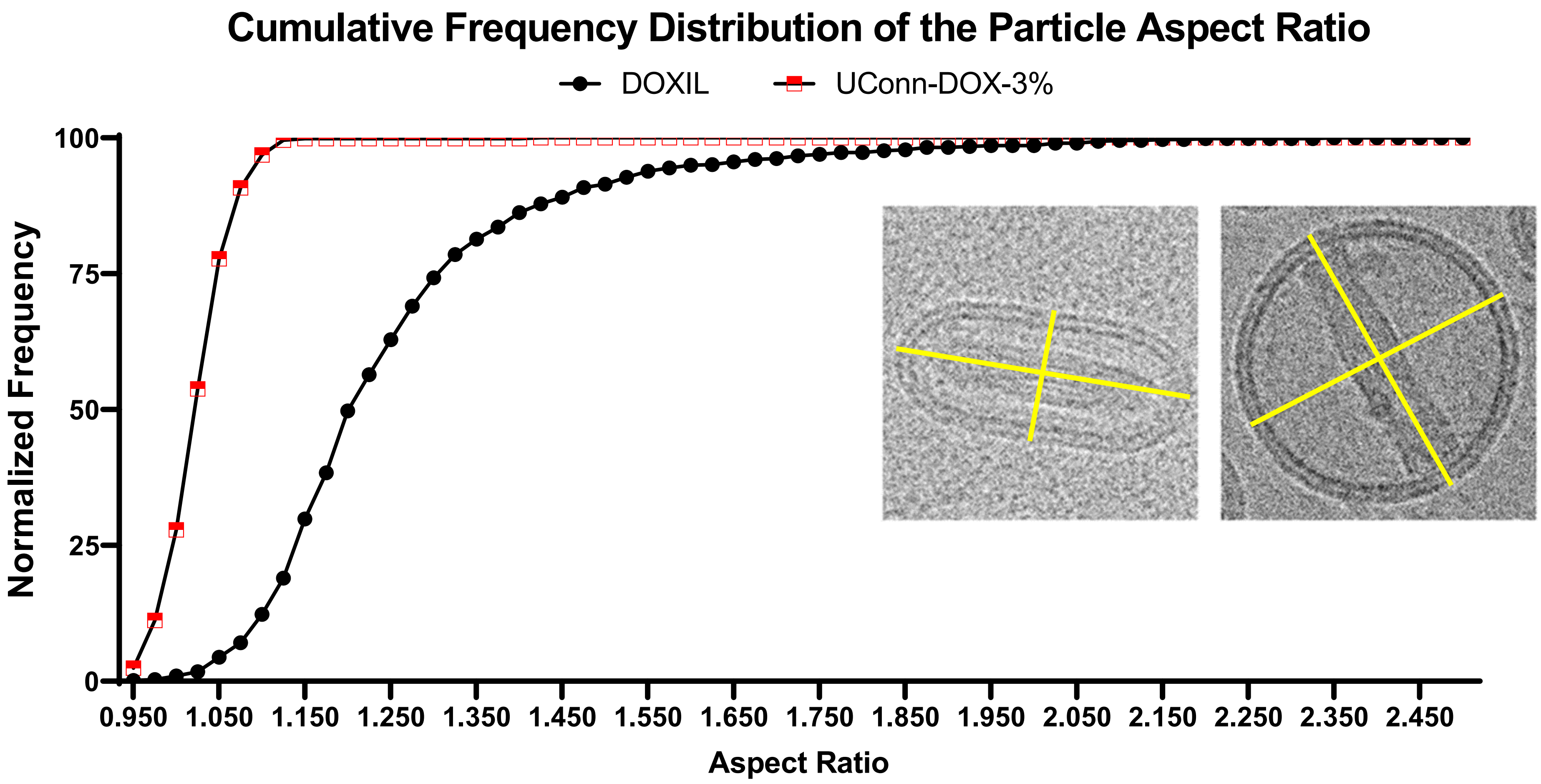
Spherical, Linear Nanocrystal

Spherical, Circular Nanocrystal

Spherical, U-Shaped Nanocrystal

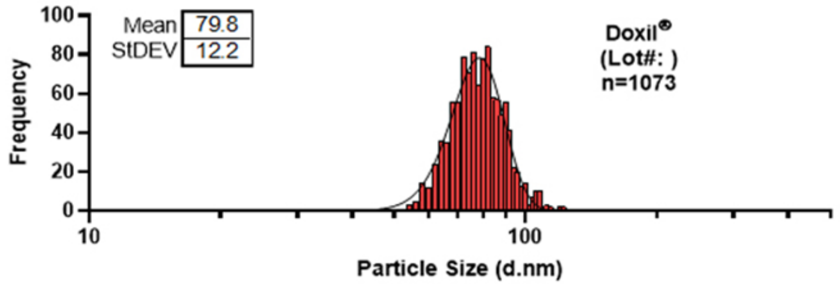
Crystal shape impact on clinical adverse events? PK Data?

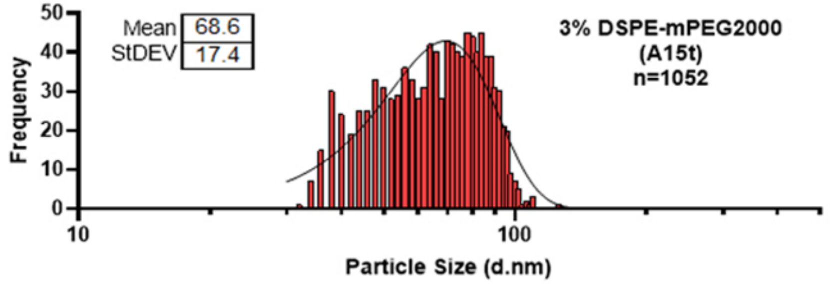
Aspect Ratio of Salt Crystal

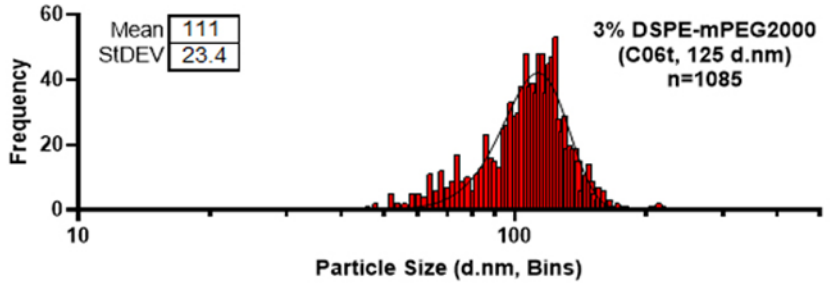


Orthogonal Sizing Techniques

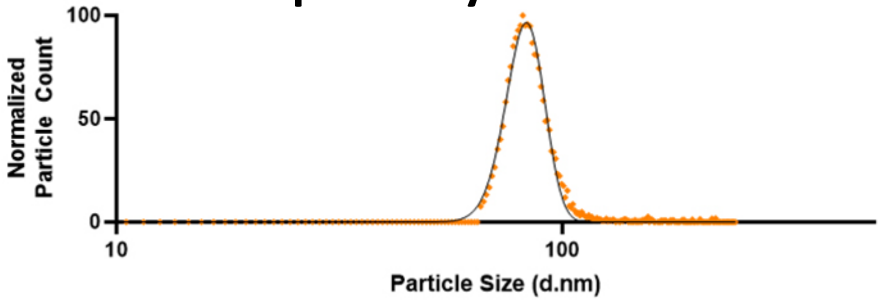
Cryo-TEM
(Number-Distribution)

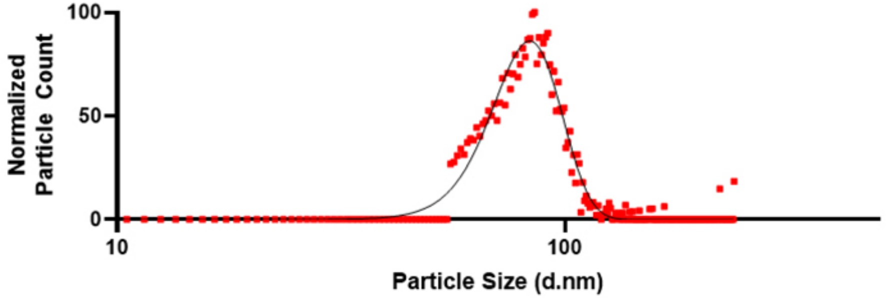


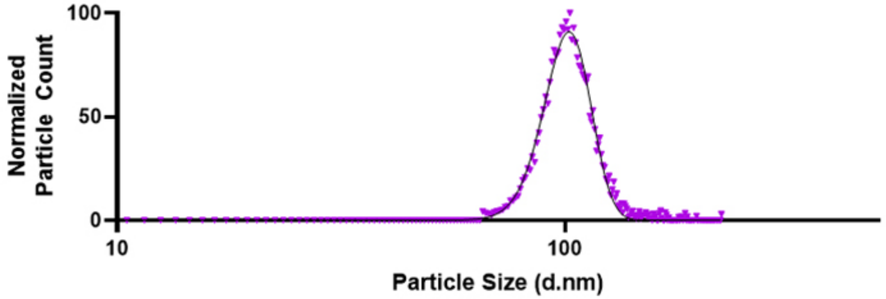




Microfluidic Resistive Pulse Sensing (Volume Distribution) Spectradyne nCS1







DLS
(Cumulant, Intensity-Based)

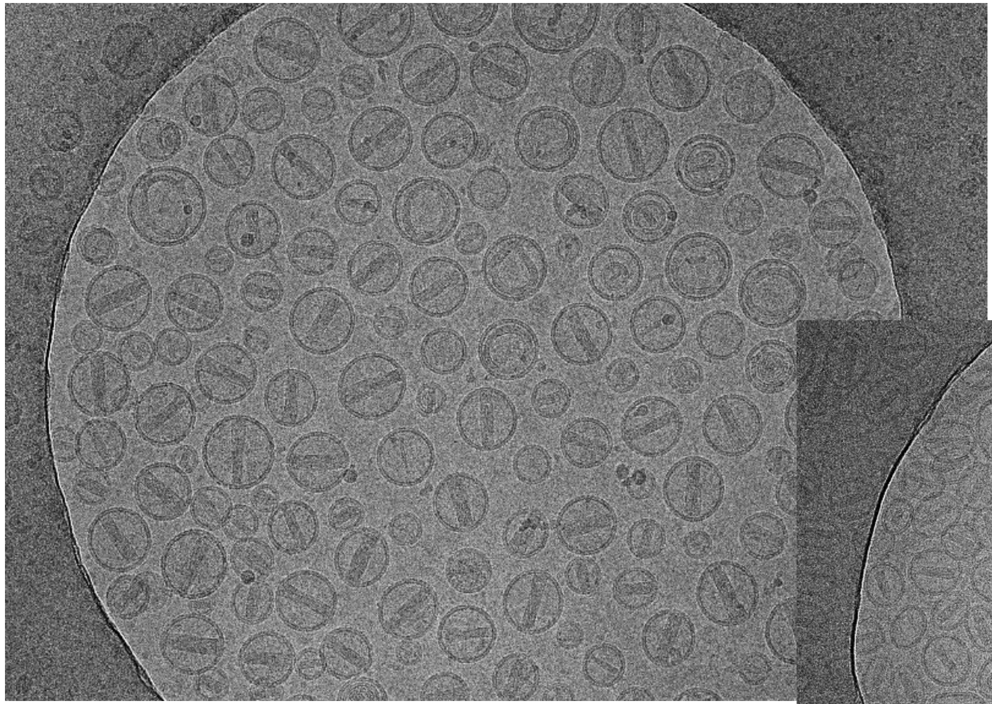
Z-average = 83.9 d.nm
PDI633 = 0.029

Z-average = 84.6 d.nm
PDI633 = 0.064

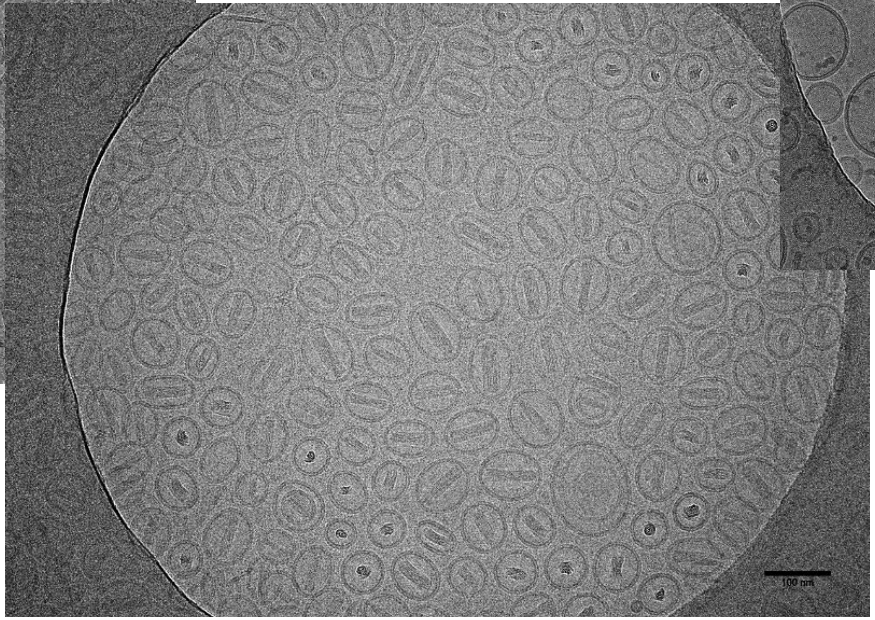
Z-average = 124.9 d.nm
PDI633 = 0.029

Particle Size: Polydispersity and Wavelength

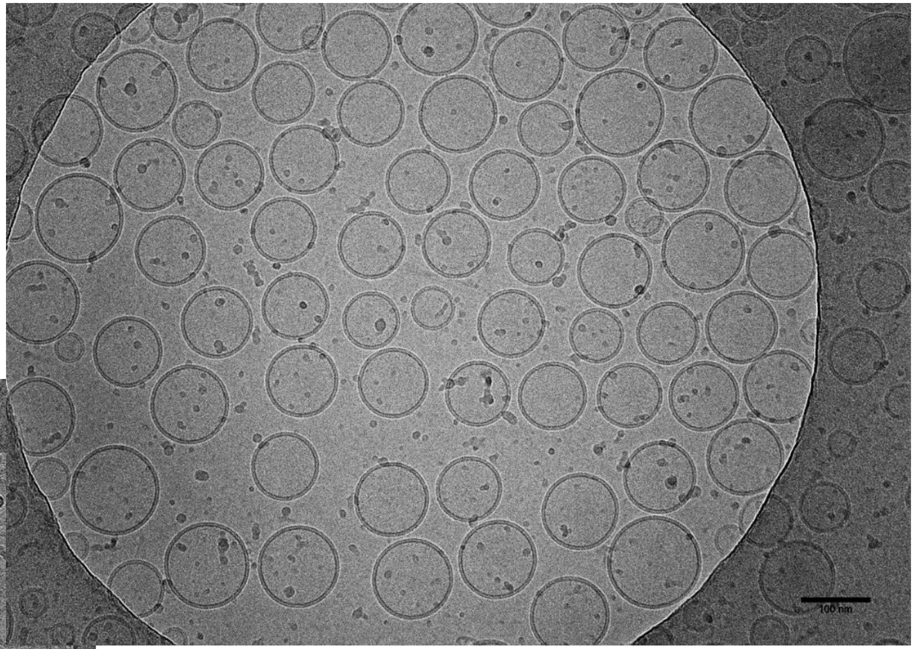
PDI_{633} = 0.064
PDI_{1300} = 0.107



PDI_{633} = 0.029
PDI_{1300} = 0.147



PDI_{633} = 0.039
PDI_{1300} = 0.008



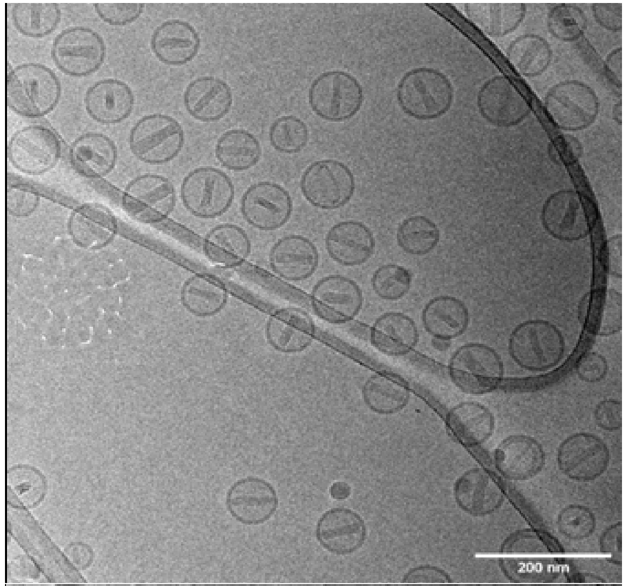
Size impacts on clinical adverse events?

Should a Cumulant PDI ≥ 0.10 be acceptable for nanoparticles?

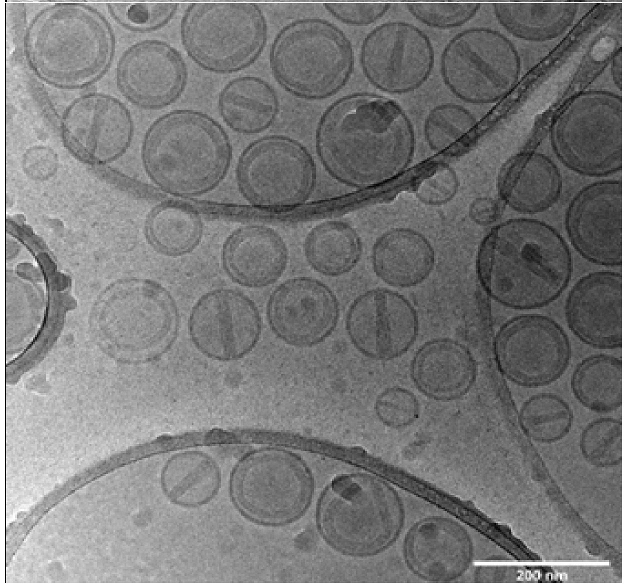
Liposomal Doxorubicin In-Vivo Study

In collaboration with: Nanotechnology Characterization Laboratory, Frederick National Laboratory for Cancer Research

Particle Characterization

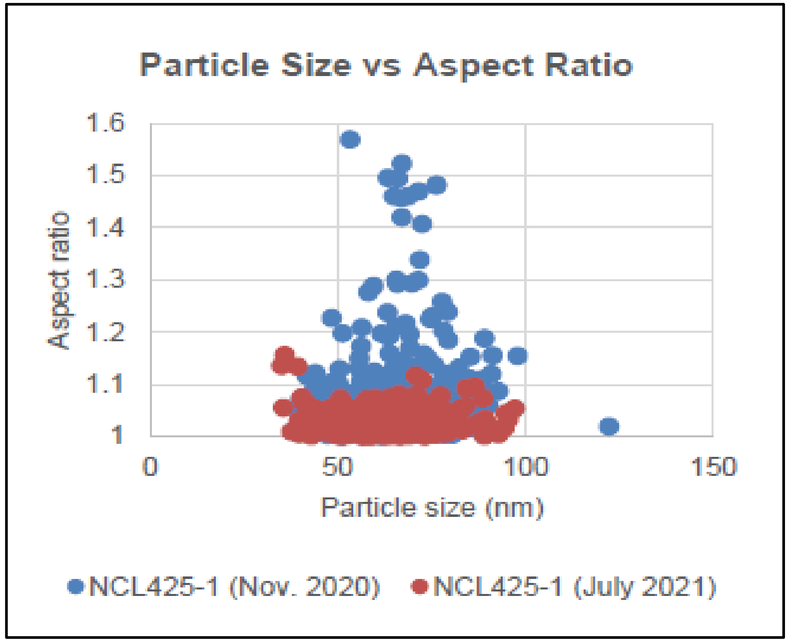


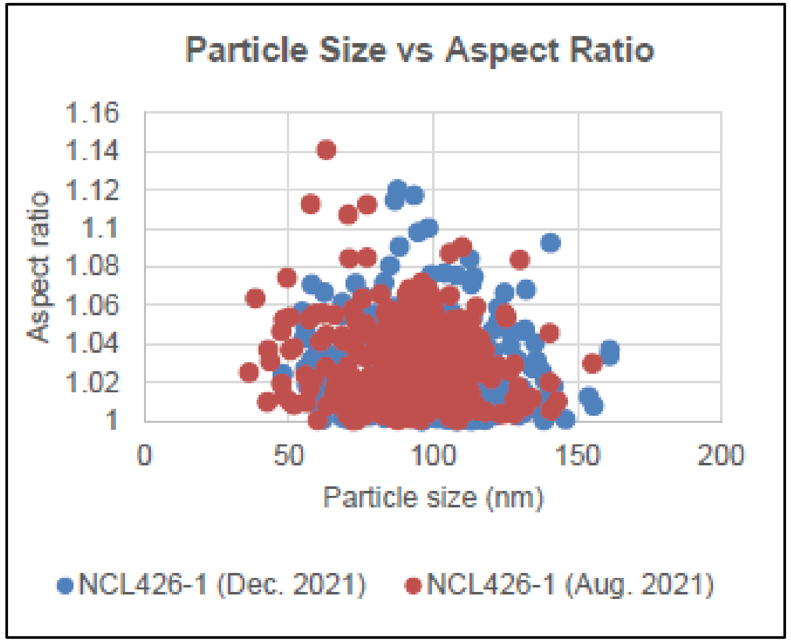
NCL425



NCL426

Sample	Avg Diameter (nm)	Aspect Ratio	Linear Crystals (%)	Circular Crystals (%)
NCL425	66.5 ± 11.8	1.09 ± 0.10	86	10
NCL426	102.6 ± 21.1	1.02 ± 0.02	42	52





In collaboration with: Nanotechnology Characterization Laboratory, Frederick National Laboratory for Cancer Research

Particle Size Characterization

Hydrodynamic Size/Size Distribution using Dynamic Light Scattering

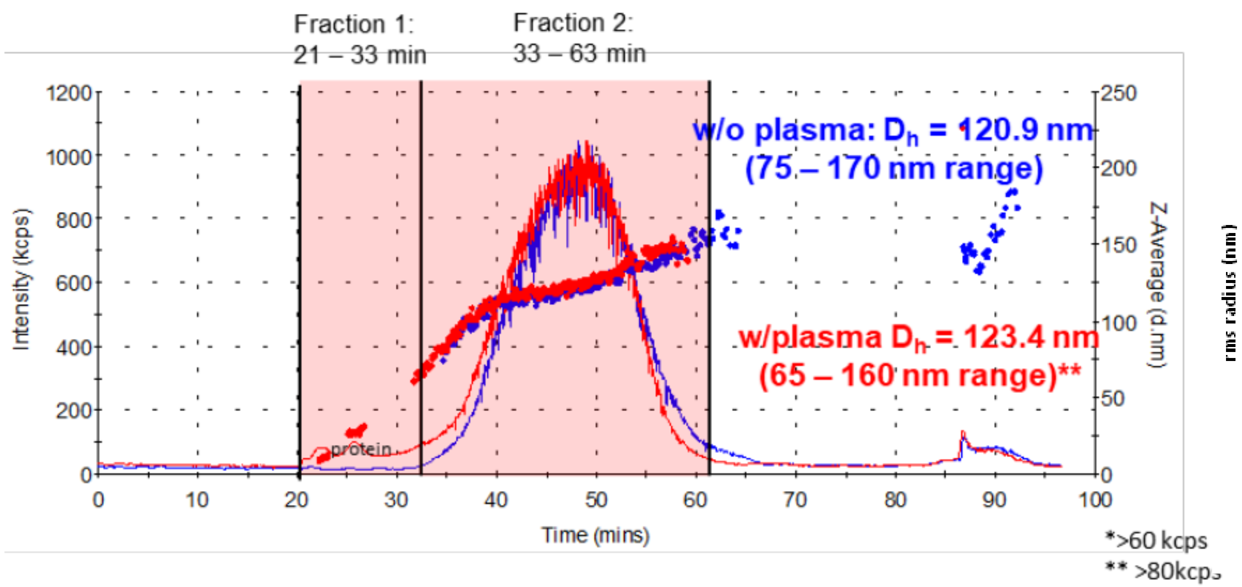
Sample	Dilution		Z-Avg (d.nm)	Int. Peak (nm)	PDI
NCL425	10 mM NaCl	100x	87.6 ± 0.6	91.3 ± 0.6	0.016 ± 0.011
		1000x	87.5 ± 0.05	91.2 ± 0.5	0.014 ± 0.006
	PBS	100x	87.3 ± 0.5	90.9 ± 0.5	0.013 ± 0.009
		1000x	87.5 ± 0.4	91.2 ± 0.5	0.018 ± 0.012
NCL426	10 mM NaCl	100x	119.5 ± 0.7	124.4 ± 0.5	0.016 ± 0.011
		1000x	119.7 ± 0.7	124.8 ± 0.7	0.015 ± 0.011
	PBS	100x	117.7 ± 0.8	123.9 ± 0.8	0.031 ± 0.017
		1000x	118.0 ± 0.6	124.7 ± 1.1	0.039 ± 0.008

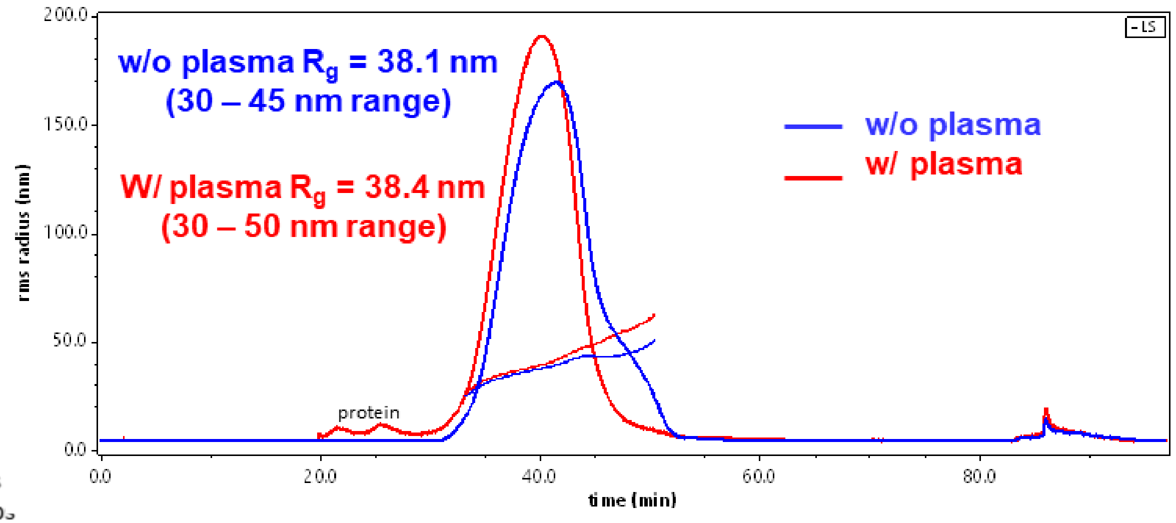
Z-Avg: intensity-weighted average. PDI: polydispersity index. Int. Peak: intensity-weighted average over the primary peak.

- NCL425 exhibited an average size of approximately 90 nm, while NCL426 had a larger size of approximately 125 nm
- The size of a well-known manufactured drug product, previously measured at approximately 90 nm, aligns with NCL425

In collaboration with: Nanotechnology Characterization Laboratory, Frederick National Laboratory for Cancer Research

Formulation Stability: NCL-426
AF4 separation with in-line MALS and DLS



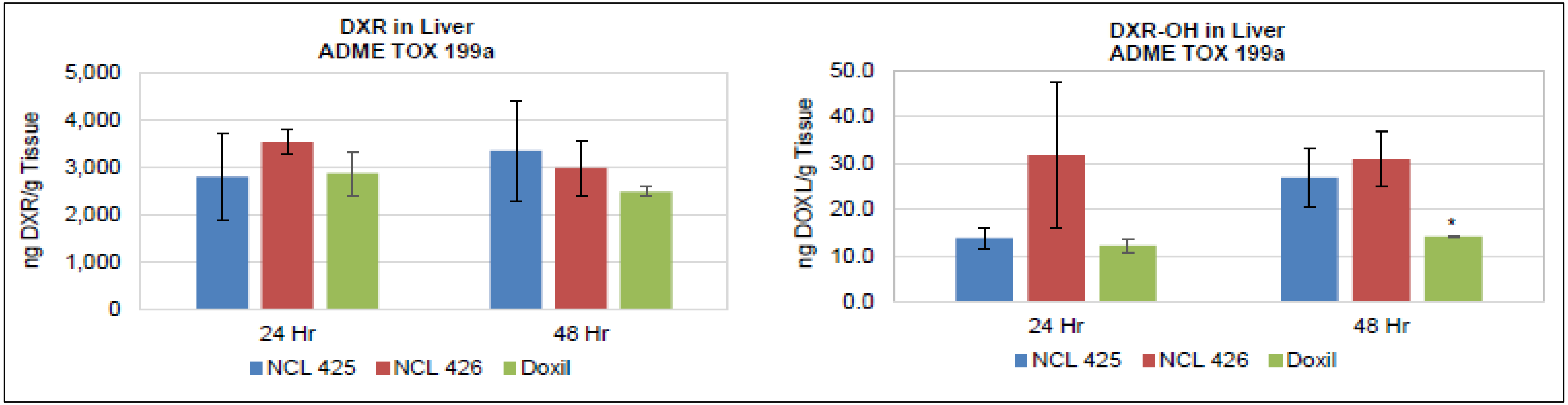


ρ = Rg/Dh, where ρ = shape factor

ρ_{NCL426} **= 1.09**
ρ_{plasma} **= 1.11**
$\rho_{Uniform\ sphere}$ **= 0.78**

Minimal shift in ρ, suggesting minimal protein binding, consistent with manufactured drug product

Toxicity Evaluation

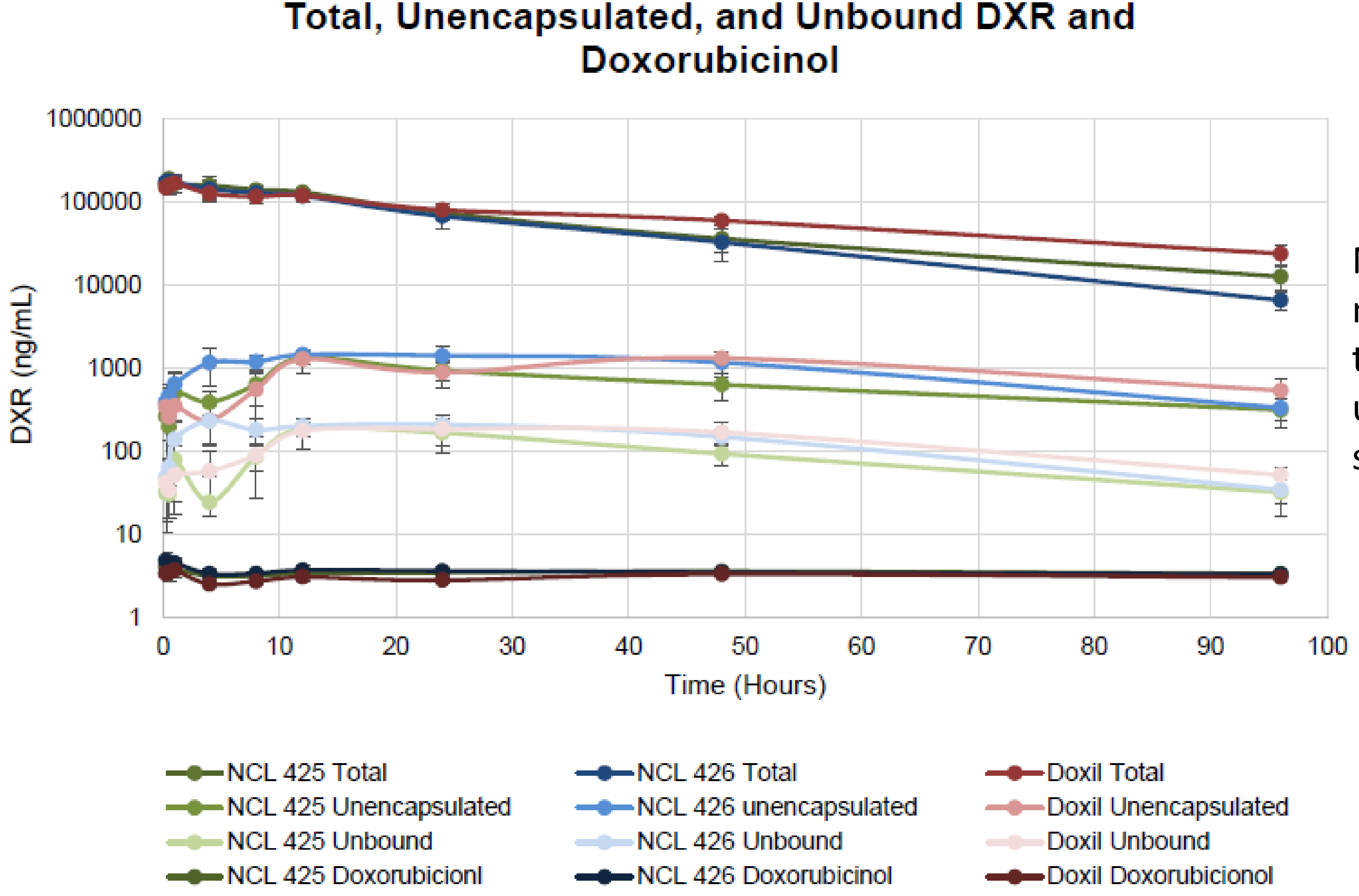


- Doxorubicin accumulation (left), and doxorubicinol accumulation (right) in the liver (Mean ± SD, N=3). *NCL426 vs. Mfd. Drug, $p<0.05$, ANOVA with Tukey's post-hoc comparisons

- Doxorubicin accumulation in the heart, liver, and ear tissue was comparable among NCL425, NCL426, and manufactured drug product.

- Statistically significant differences were found in doxorubicinol concentrations in the heart and liver, indicating slight variations in tissue concentration profiles.

Bioequivalence: In Vivo Drug Release



NCL425, NCL426, and manufactured drug product, total, unencapsulated and unbound drug profiles were similar in vivo.

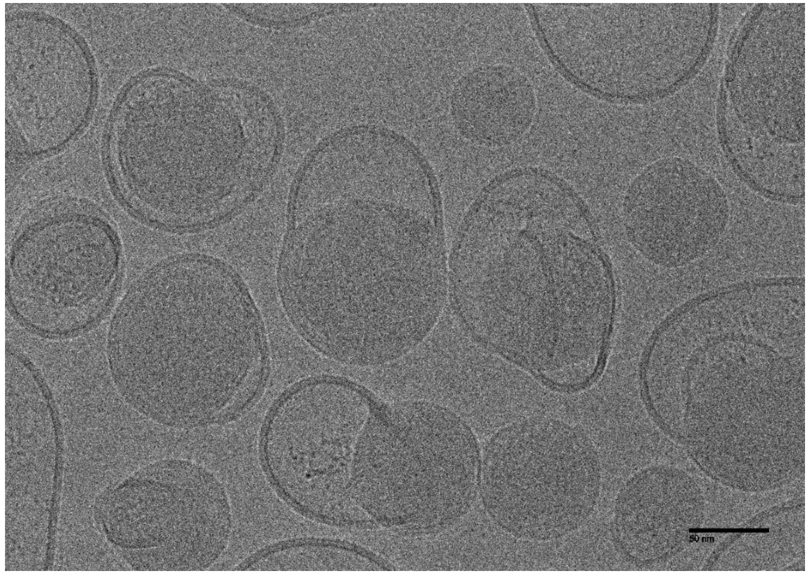
In collaboration with: Nanotechnology Characterization Laboratory, Frederick National Laboratory for Cancer Research

mRNA-LNPs

Structural Properties

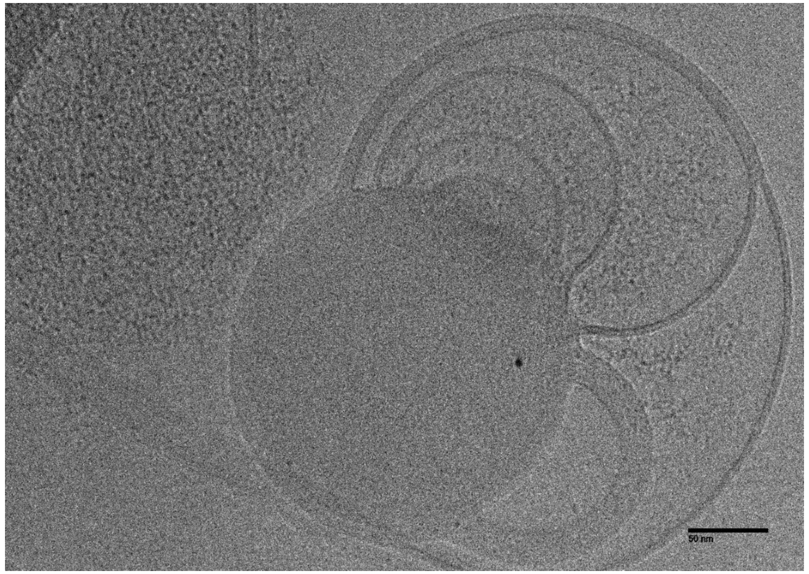
mRNA-LNPs and formulation stability

Low Phase Transition Lipid , 1.7% EtOH



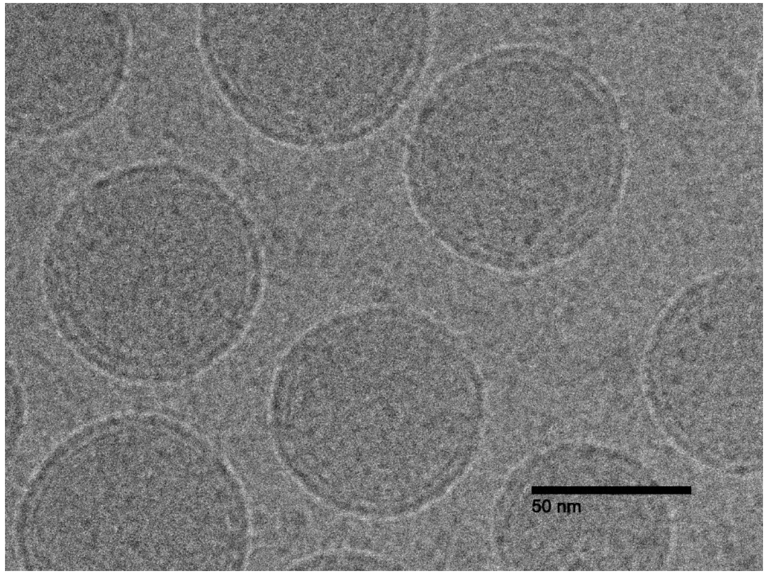
Bleb-like structure
Possible mRNA separation into multiple compartments

Low Phase Transition Lipid , 5.0% EtOH



Bleb-like structure, swelling, lamella extended
Possible mRNA separation into multiple compartments

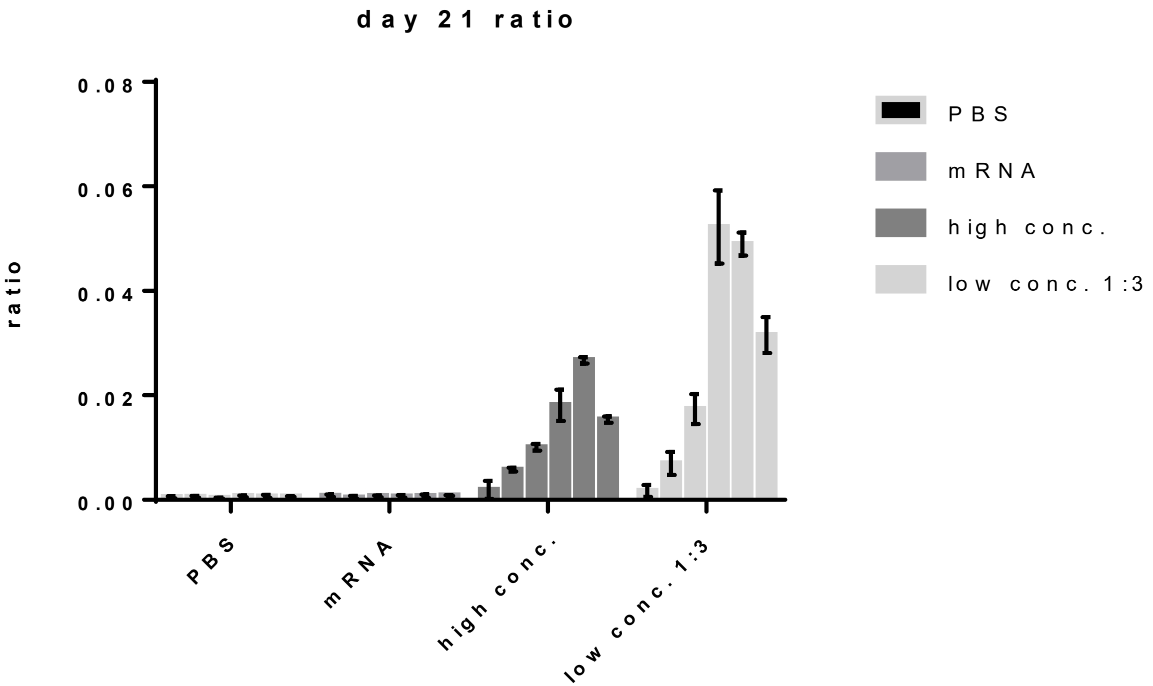
Higher phase transition lipids, ethanol <0.5%

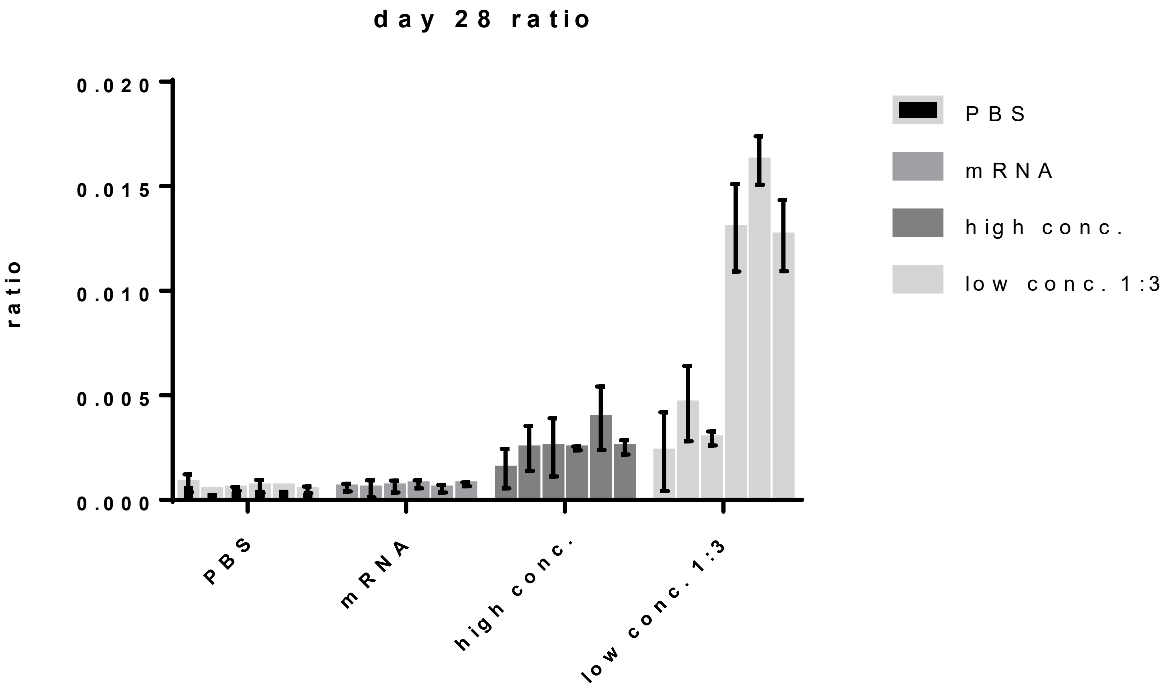


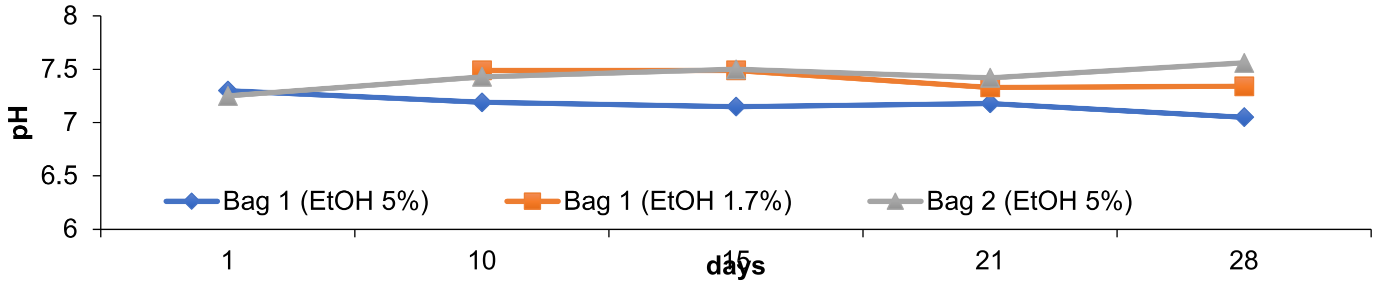
Solid-core and no separation, more stable structure

mRNA LNP Transfection, 28 days

Particles: SSOP-POPE-Cholesterol-GM-PEG2k
Cell line: K562, Chronic Myelogenous Leukemia Cells

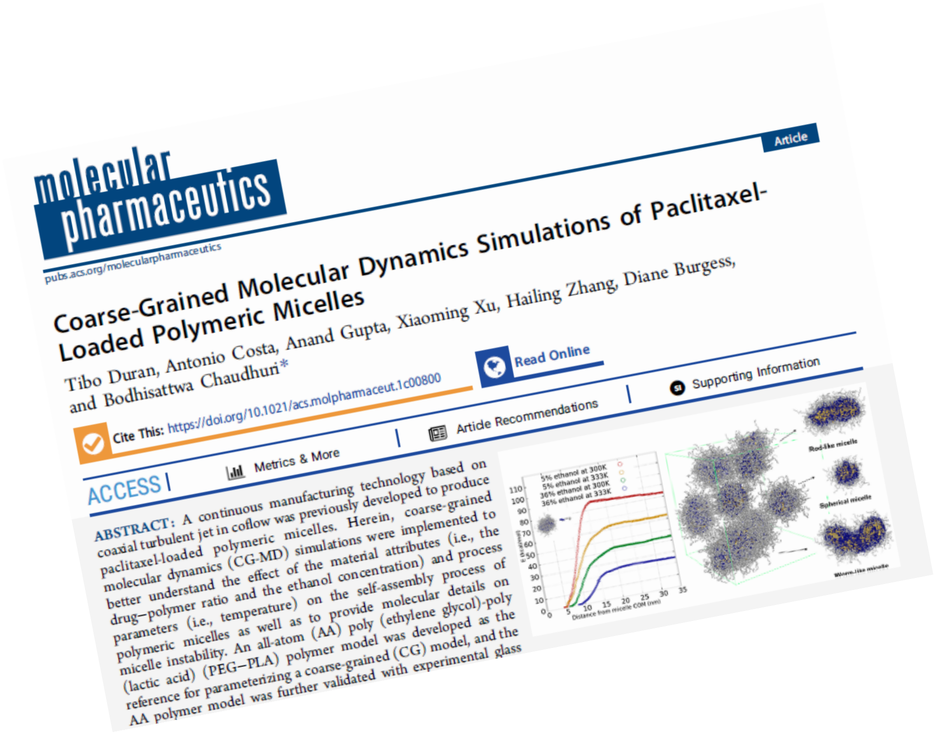






Computational Fluid Dynamics and Molecular Dynamics (CG-MD)

Work performed by Dr. Bodhi Chaudhuri's Lab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Article

Coarse-Grained Molecular Dynamics Simulations of Paclitaxel-Loaded Polymeric Micelles

Tibo Duran, Antonio Costa, Anand Gupta, Xiaoming Xu, Hailing Zhang, Diane Burgess, and Bodhisattwa Chaudhuri*

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Cite This: https://doi.org/10.1021/acs.molpharmaceut.1c00800
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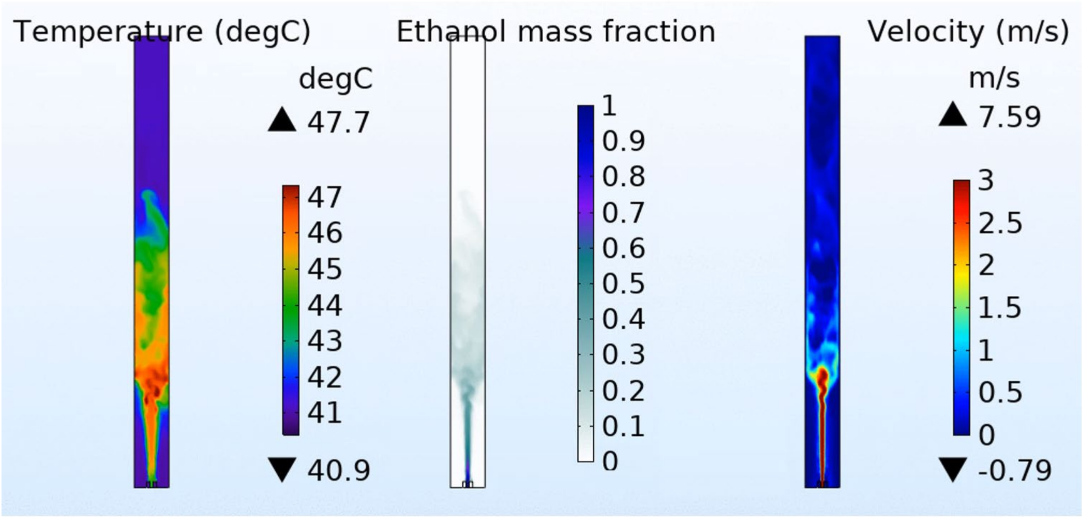
ABSTRACT: A continuous manufacturing technology based on coaxial turbulent jet in coflow was previously developed to produce paclitaxel-loaded polymeric micelles. Herein, coarse-grained molecular dynamics (CG-MD) simulations were implemented to better understand the effect of the material attributes (i.e., the drug–polymer ratio and the ethanol concentration) and process parameters (i.e., temperature) on the self-assembly process of polymeric micelles as well as to provide molecular details on micelle instability. An all-atom (AA) poly (ethylene glycol)-poly (lactic acid) (PEG–PLA) polymer model was developed as the reference for parameterizing a coarse-grained (CG) model, and the AA polymer model was further validated with experimental glass

CFD Jet-flow Studies

Simulation
COMSOL MULTIPHYSICS (V 5.4), LES model.



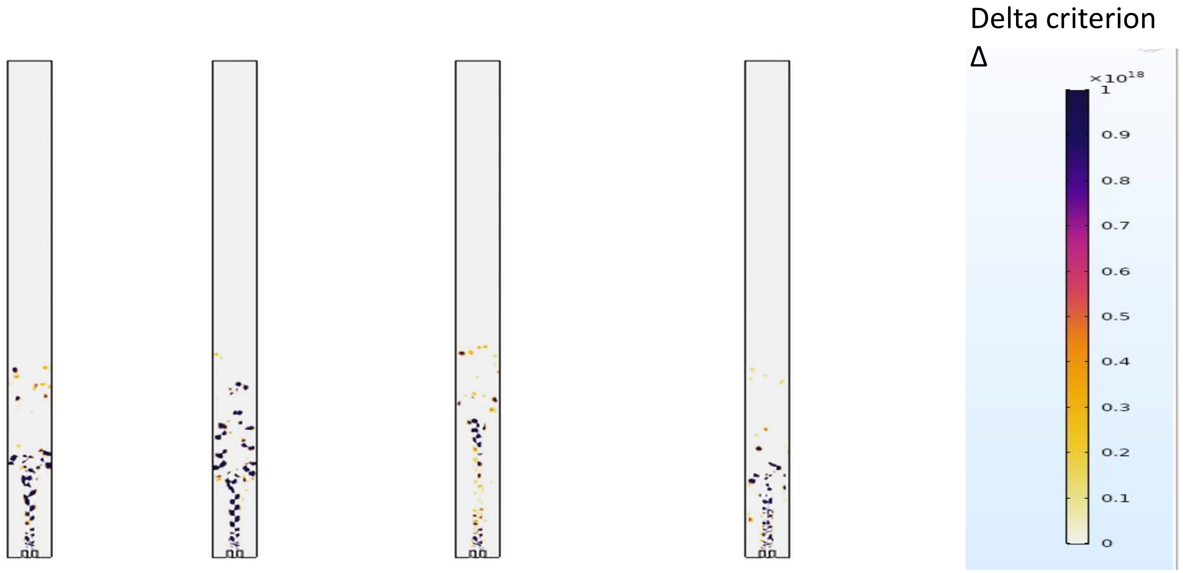
Experimental
Track jet flow with blue dye in ethanol phase

Eddy Current Analysis (Below)

Delta criterion defines vortices as regions in the value of delta is greater than 0.

$$\Delta = \frac{Q^3}{27} + \frac{R^2}{4} > 0$$

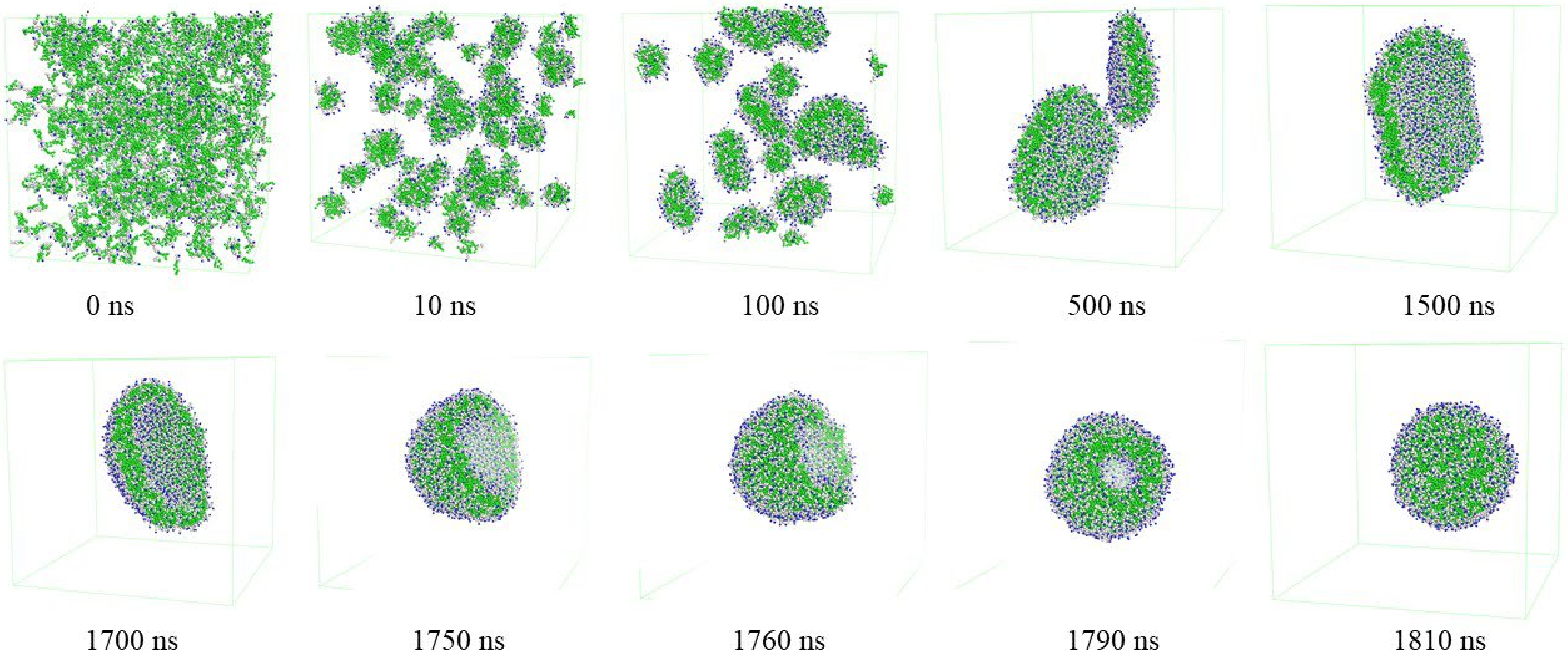
Ω_{ij}: rotation rate tensor
S_{ij}: strain rate tensor



100 mL/min 33.6 °C | 100 mL/min 58 °C | 150 mL/min 59.8 °C | 200 mL/min 59.8 °C

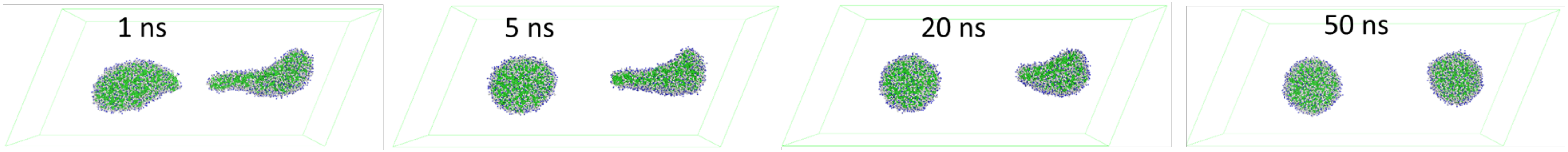
Simulations provide additional information that is not measurable or difficult to measure.

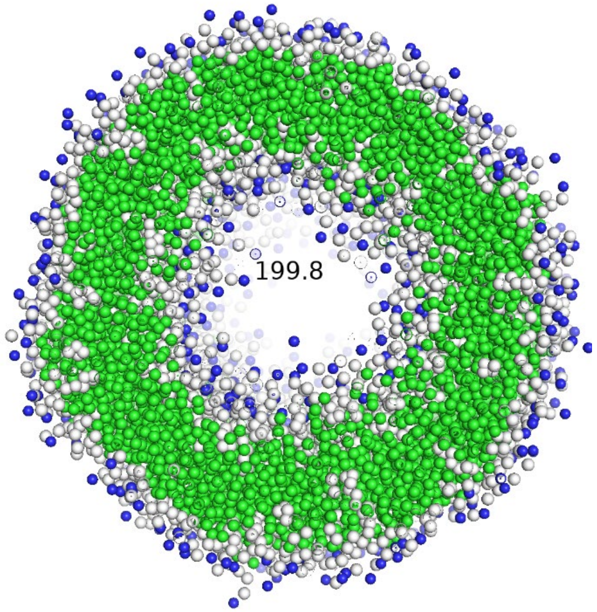
MD Liposome Formation Studies



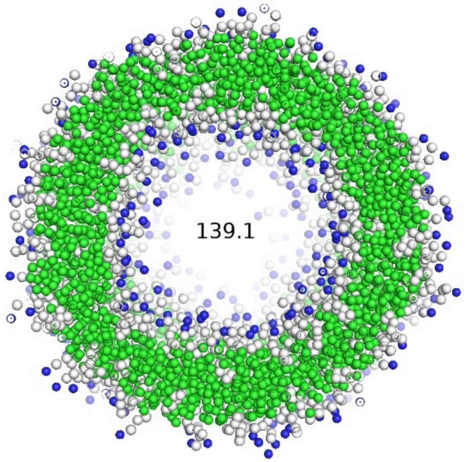
Simulation temperature: 333 K

Shear Simulations (Case v_s = 0.025 nm/ps)

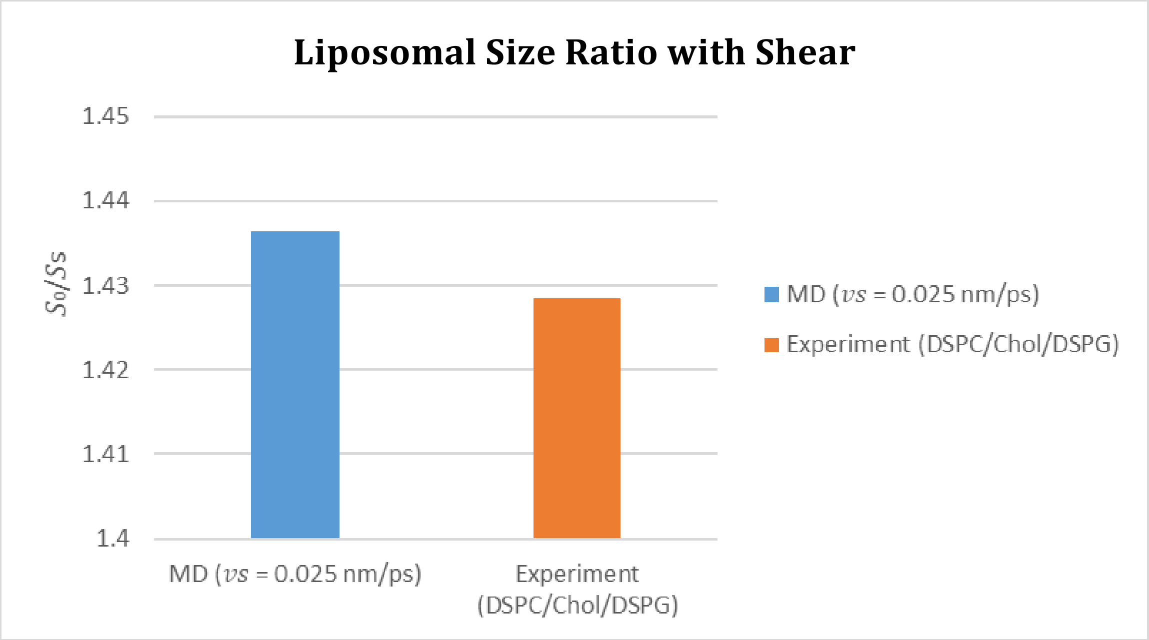




Before shear (19.98 nm)



After shear (13.91 nm)

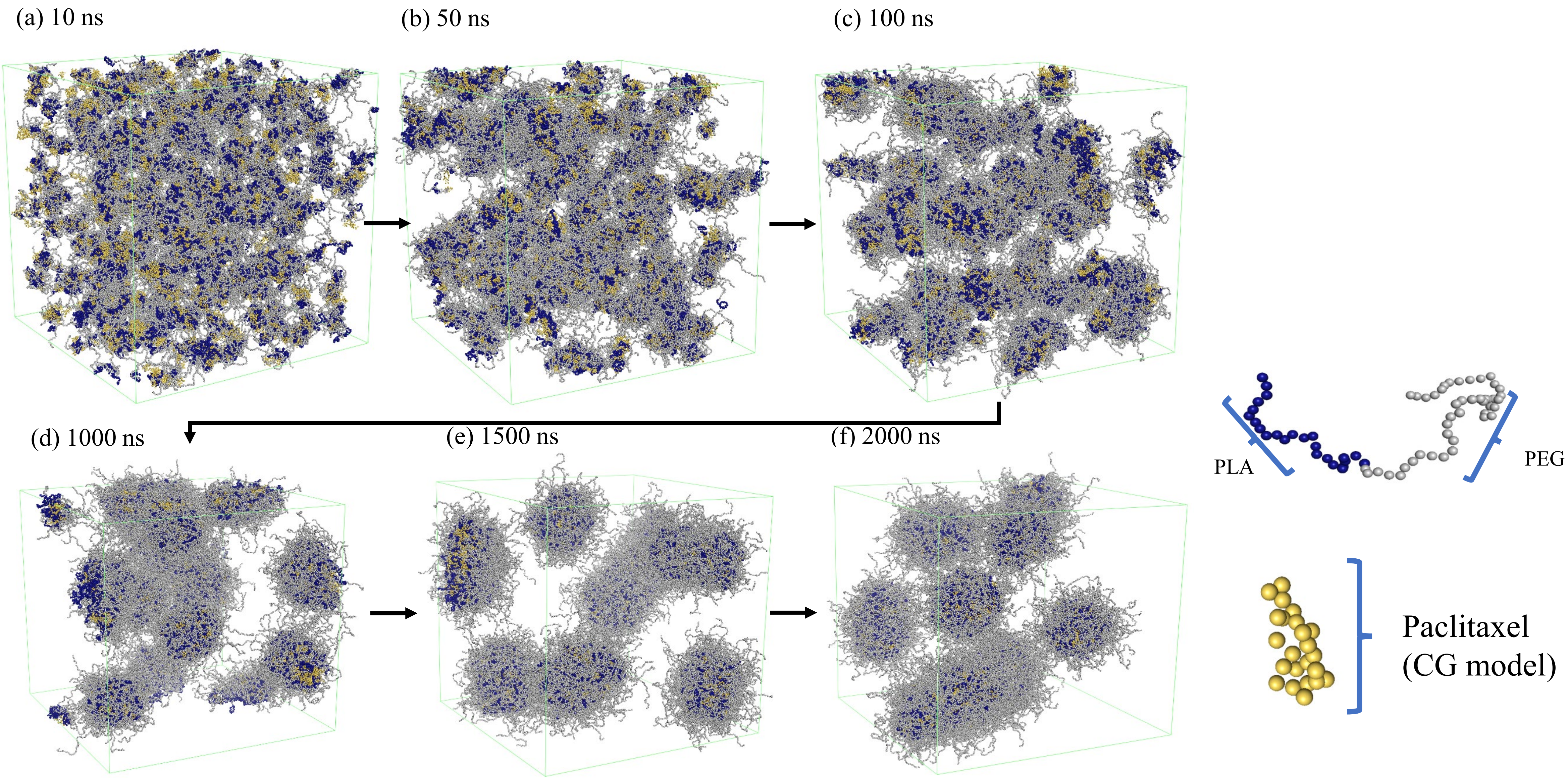


Liposome size ratio	Molecular Dynamics simulation results	Experiment results
$S0/Ss$	1.436	1.428

$$S_0 = liposome\ size\ before\ shear$$
$$S_s = liposome\ size\ after\ shear$$

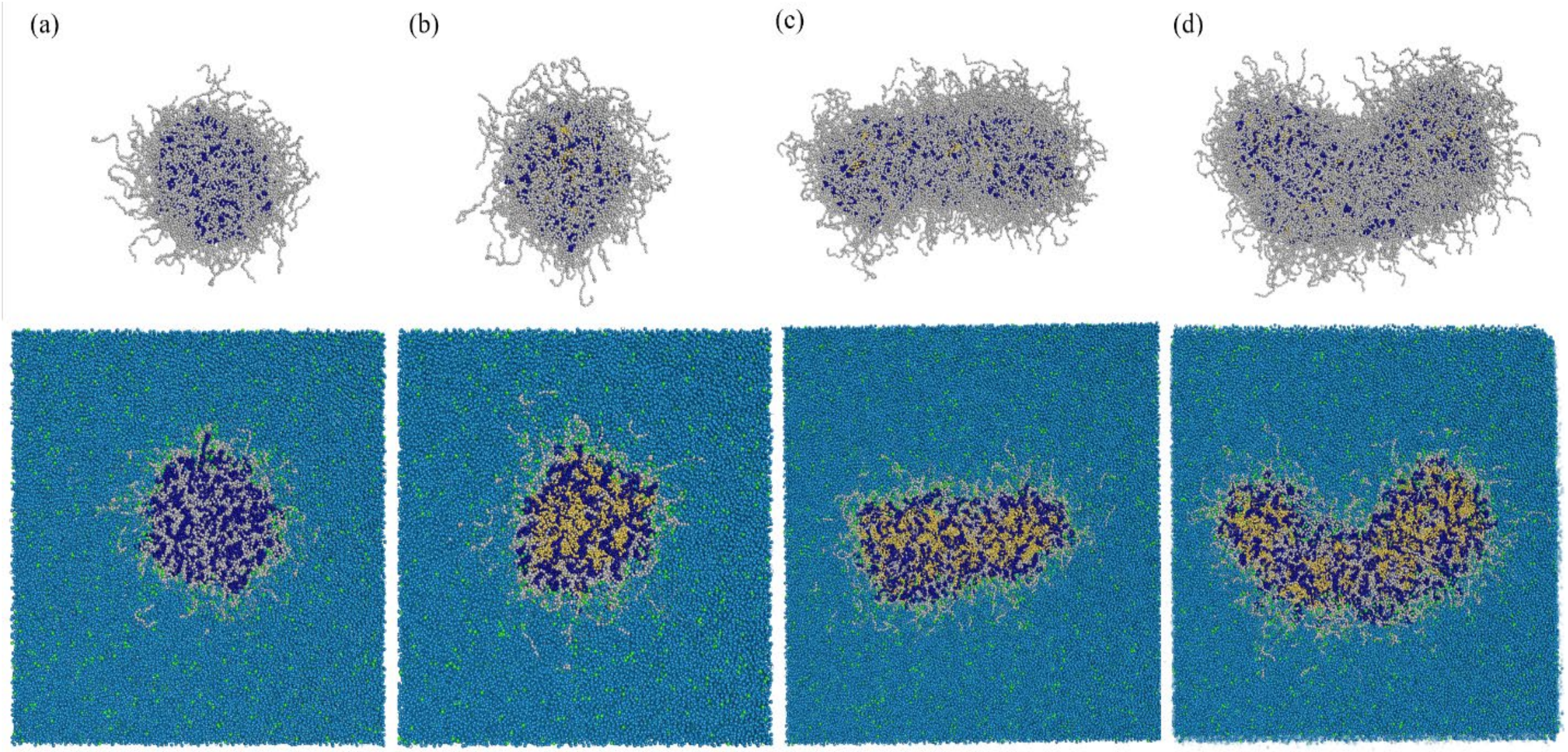
- Higher shear rates resulted in smaller liposome formation
- MD issue is actual particle size due to constraints

Paclitaxel PEG-PLA Micelle Formation



Tibo Duran, Antonio Costa, Anand Gupta, Xiaoming Xu, Hailing Zhang, Diane Burgess, and Bodhisattwa Chaudhuri, Molecular Pharmaceutics 2022 19 (4), 1117-1134, DOI: 10.1021/acs.molpharmaceut.1c00800

Polymeric Micelle Structures

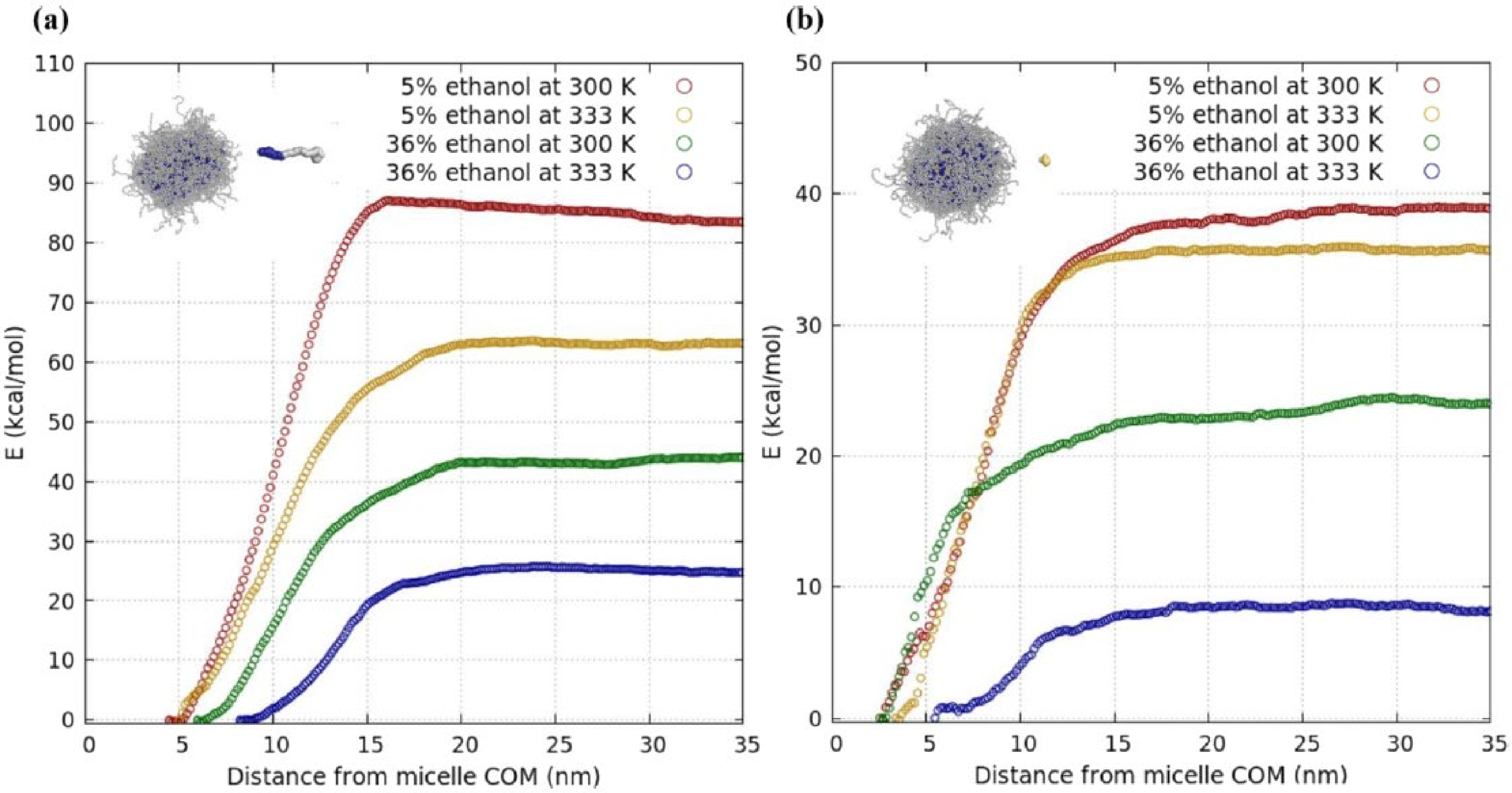


(a) blank spherical structures, (b) drug-loaded spherical structures, (c) drug-loaded rod-like structure, (d) drug-loaded worm-like structure

PLA beads, PEG beads, PTX beads, water beads, and ethanol beads

Tibo Duran, Antonio Costa, Anand Gupta, Xiaoming Xu, Hailing Zhang, Diane Burgess, and Bodhisattwa Chaudhuri, Molecular Pharmaceutics 2022 19 (4), 1117-1134, DOI: 10.1021/acs.molpharmaceut.1c00800

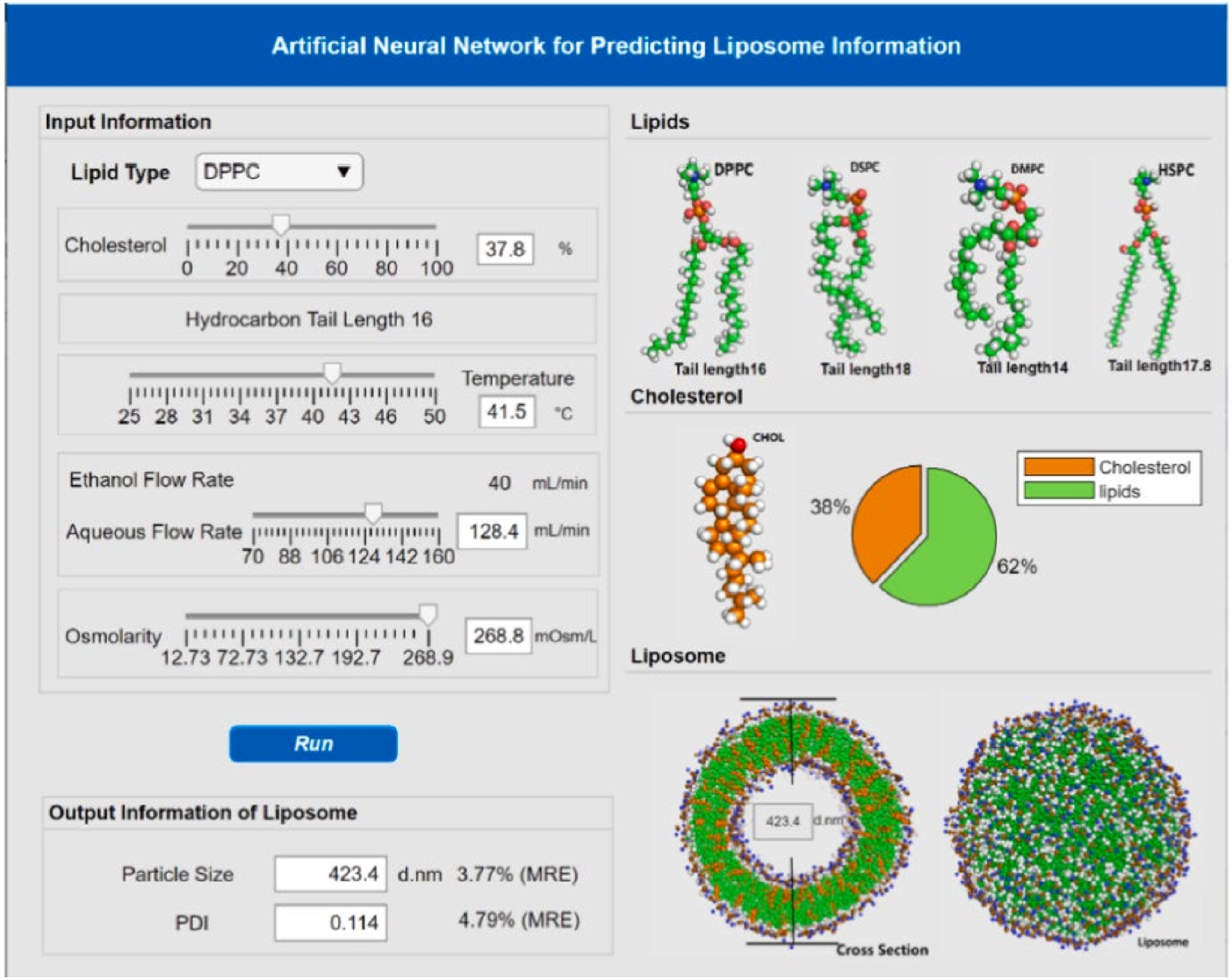
Free Energy of Polymeric Micelle Dissociation

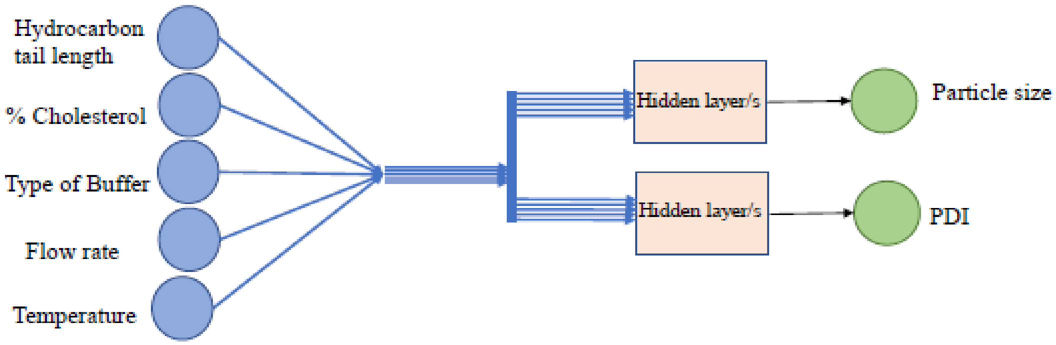


Potentials of the mean force calculated along the reaction coordinates for (a) pulling single PEG−PLA and (b) pulling single paclitaxel molecules away from the COM of the rest of the aggregate.

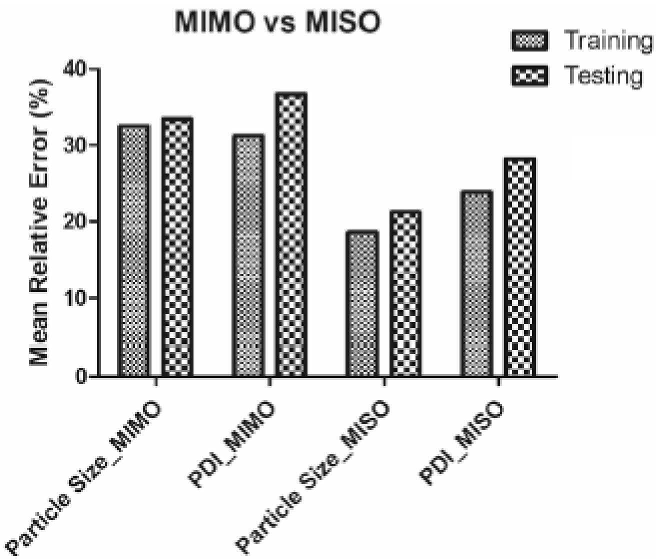
Tibo Duran, Antonio Costa, Anand Gupta, Xiaoming Xu, Hailing Zhang, Diane Burgess, and Bodhisattwa Chaudhuri, Molecular Pharmaceutics 2022 19 (4), 1117-1134, DOI: 10.1021/acs.molpharmaceut.1c00800

Artificial neural networks for continuous manufacturing





Compared multi-input multi-output model (MIMO) and multi-input single-output model (MISO) models. MIMO vs MISO for predicting liposome particle size and PDI.



S. Sansare, T. Duran, H. Mohammadiarani, M. Goyal, G. Yenduri, A. Costa, et al., Artificial neural networks in tandem with molecular descriptors as predictive tools for continuous liposome manufacturing, Int J Pharm, 603 (2021), Article 120713

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- 75F40120C00201: Continuous Processing of Liposomal Nanoparticles as Reference Materials for Drug Product (2020-2022)
- U01FD006975: Continuous Manufacturing of Nanoparticles: Establishing Real-Time-Release Testing Methods for a GMP-Ready System and Evaluation of Liposomal Morphological Changes in Real-Time (2020-2022)