U.S. FOOD & DRUG ADMINISTRATION

Advanced Separations and Detection in Assessment of Quality for Drug Products Containing Nanomaterials

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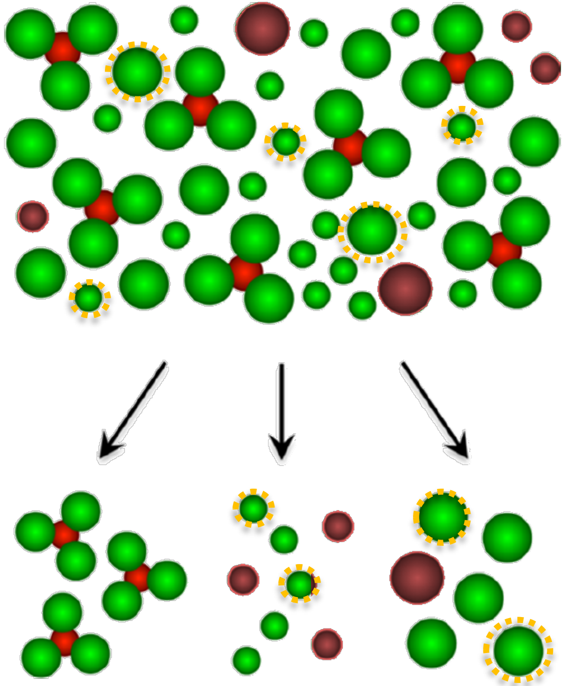
CDER | US FDA

– [2023 NanoDay Symposium: Continuous Manufacturing of Nanomaterials] –
October 11, 2023

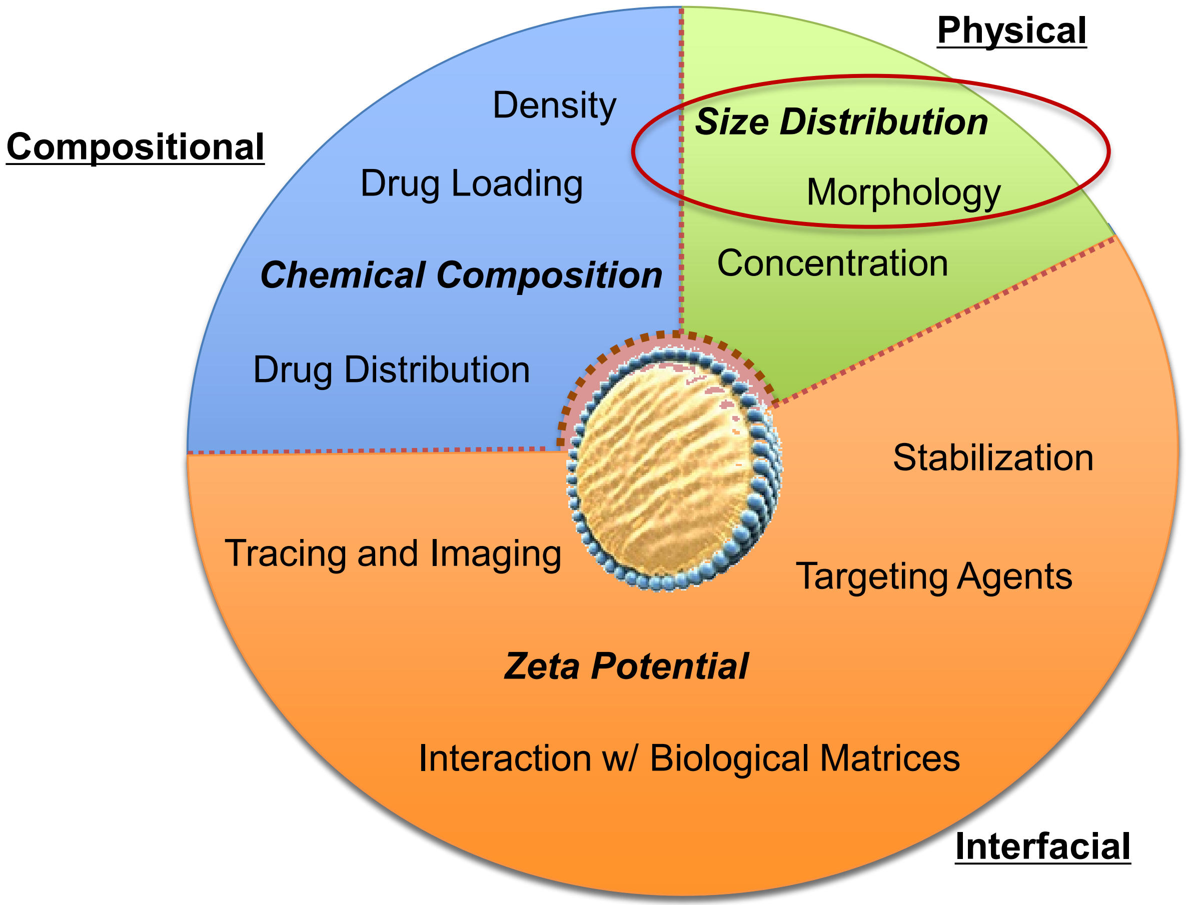
Complexities of Products Containing Nanomaterials

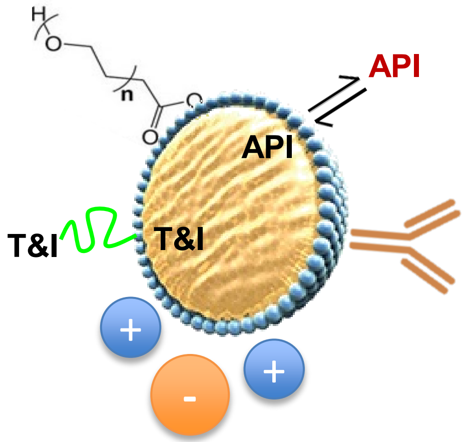


"Determining average *properties* may <u>not be sufficient</u> for products with <u>multiple concurrent distributions</u> of *properties*"

Stavis, S. M.; Fagan, J. A.; Stopa, M.; Liddle, J. A. Nanoparticle Manufacturing – Heterogeneity through Processes to Products. *ACS Appl. Nano Mater.* **2018**, DOI: 10.1021/acsanm.8b01239.

When Simple is Complicated…





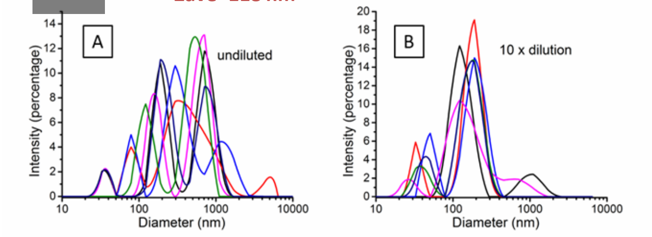
"As nanomaterials become more complex, how do we address the *simple* questions"

Complementary Size Measurement (Cyclosporine Ophthalmic Emulsions)

Research results provided pivotal support to the development of PSG and approval of first generics!

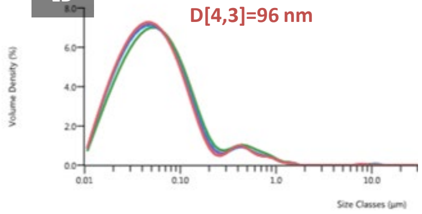
DLS

Zave=118 nm



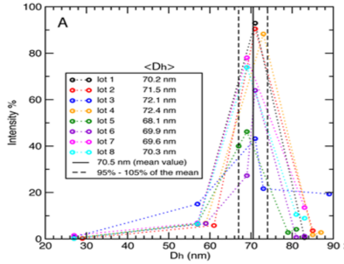
LD

D[4,3]=96 nm



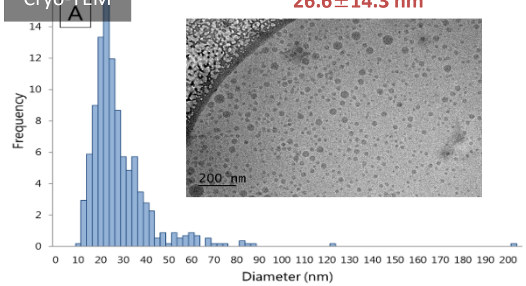
DOSY NMR

mean=70.5 nm



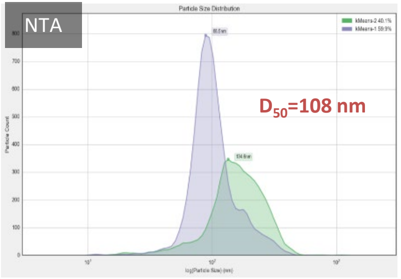
Cryo-TEM

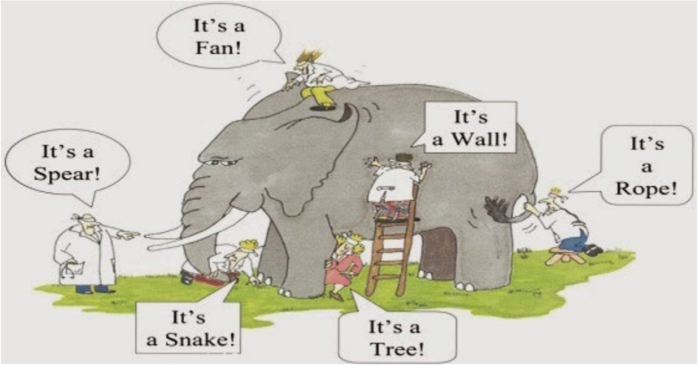
26.6±14.3 nm



NTA

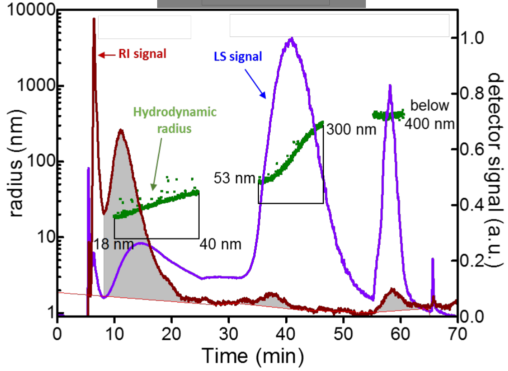
D_{50}=108 nm





https://medium.com/betterism/the-blind-men-and-the-elephant-596ec8a72a7d

AF4+MALS+DLS+RI

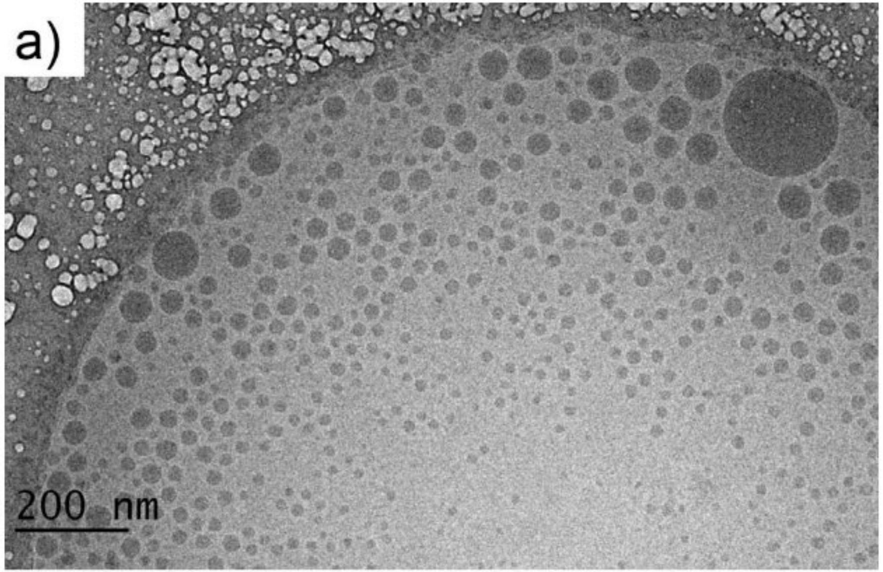


1st: 30 - 80 nm (87.8%); 2nd: 100 – 600 nm (5.3%)

P. Petrochenko, N. Pavurala, Y. Wu, S. Y Wong, H. Parhiz, K. Chen, S.M. Patil, H. Qu, P. Buoniconti, A. Mohammad, S. Choi, D. Kozak, M. Ashraf, C.N. Cruz, J. Zheng, X. Xu. Analytical Considerations for Measuring the Globule Size Distribution of Cyclosporine Ophthalmic Emulsions. International Journal of Pharmaceutics (2018). 550(1-2), 229-239.

GSD for Cyclosporin Ophthalmic Emulsions

First time *use of AF4 in characterization of a finished drug product to support BE determination*.



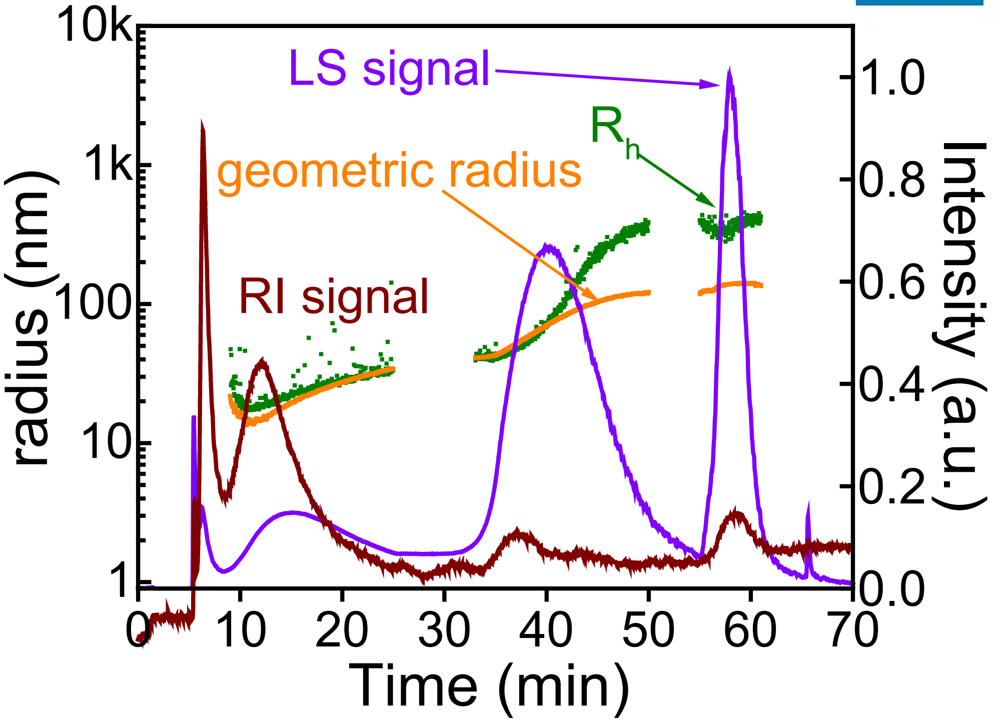


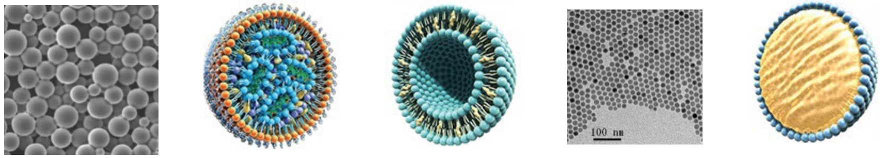
Fig. 4. AF4-MALS fractogram of Restasis® and the hydrodynamic (D_h) and geometric size (D_g) information. MALS signal at 90° and RI signal are shown. D_g and D_h were plotted using the same scale.

H. Qu, J. Wang, Y. Wu, J. Zheng, Y.S.R. Krishnaiah, M. Absar, S. Choi, M. Ashraf, C.N. Cruz and X. Xu. Asymmetric Flow Field Flow Fractionation for the Characterization of Globule Size Distribution in Complex Formulations: A Cyclosporine Ophthalmic Emulsion Case. International Journal of Pharmaceutics. (2018). 538(1-2), 215-222.

Separation and Characterization of Complex Products

What sample can I analyze?

Nanoparticles



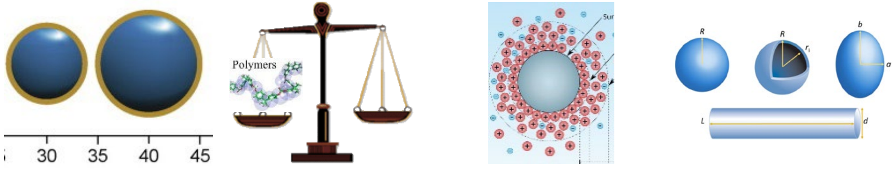
PLGA | Lipid NPs | Liposome | Colloidal Iron | Emulsion

Biotherapeutics

Polymers

Therapeutic protein/peptide | Antibody-drug conjugate | Nucleotide | API/Excipient

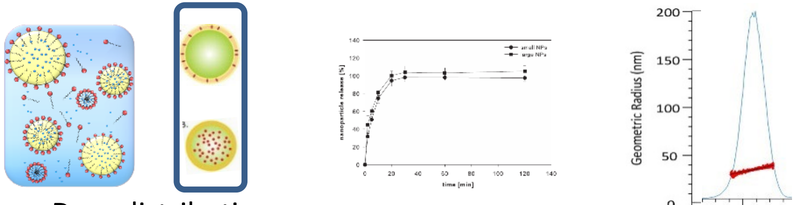
What properties can I determine?



Particle size | Molecular mass | Surface charge | Conformation

What information can I obtain?

Aggregation status | Formulation stability | Conjugation efficiency



Drug distribution | Drug release | Size distribution

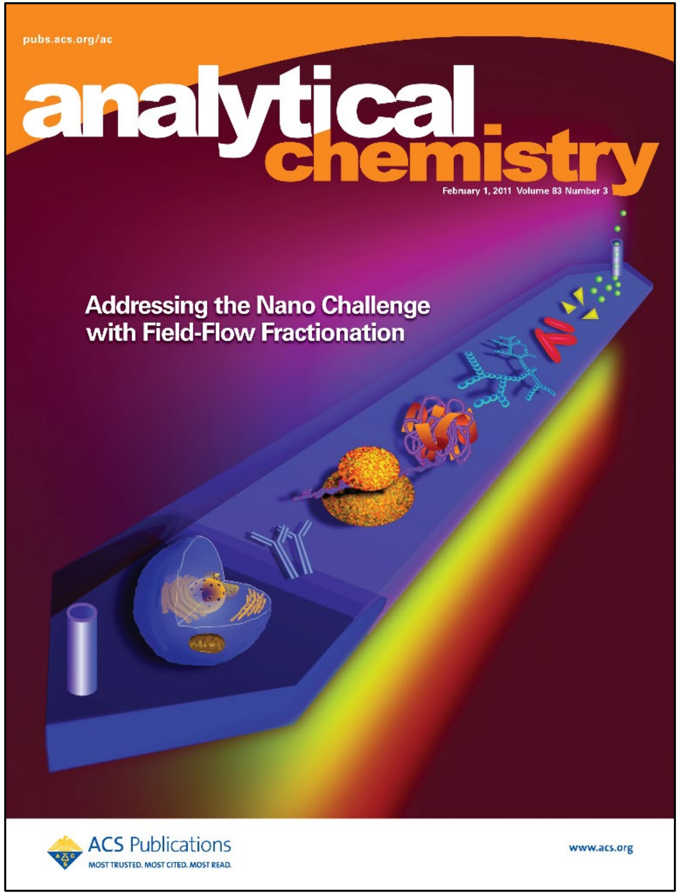
Asymmetrical Flow FFF

Thermal FFF

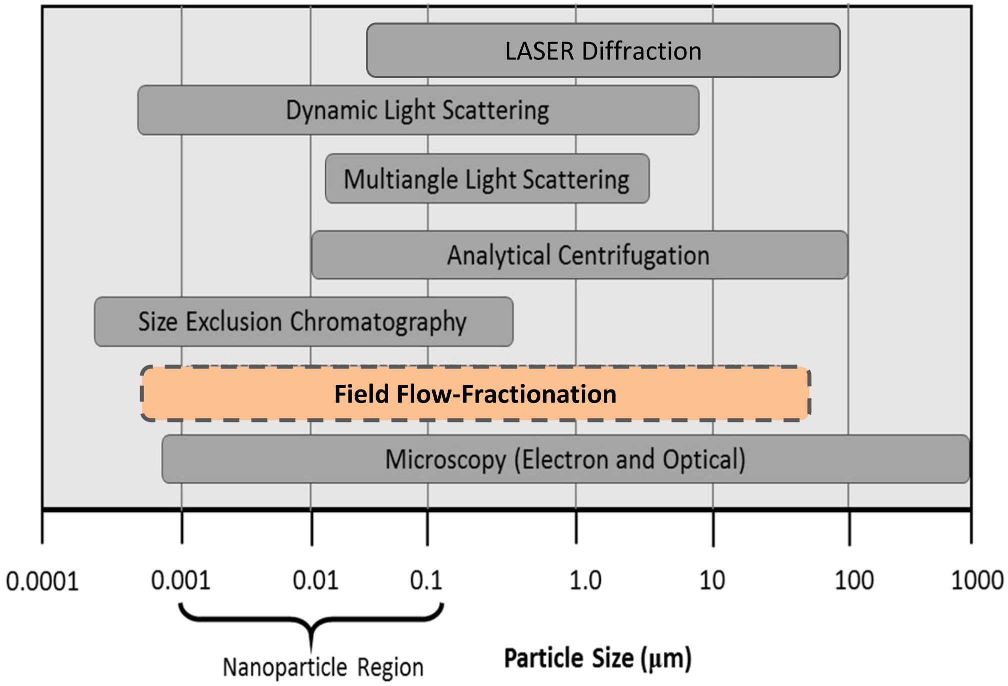
IC & CE

2D chromatography

Field-Flow Fractionation (FFF)

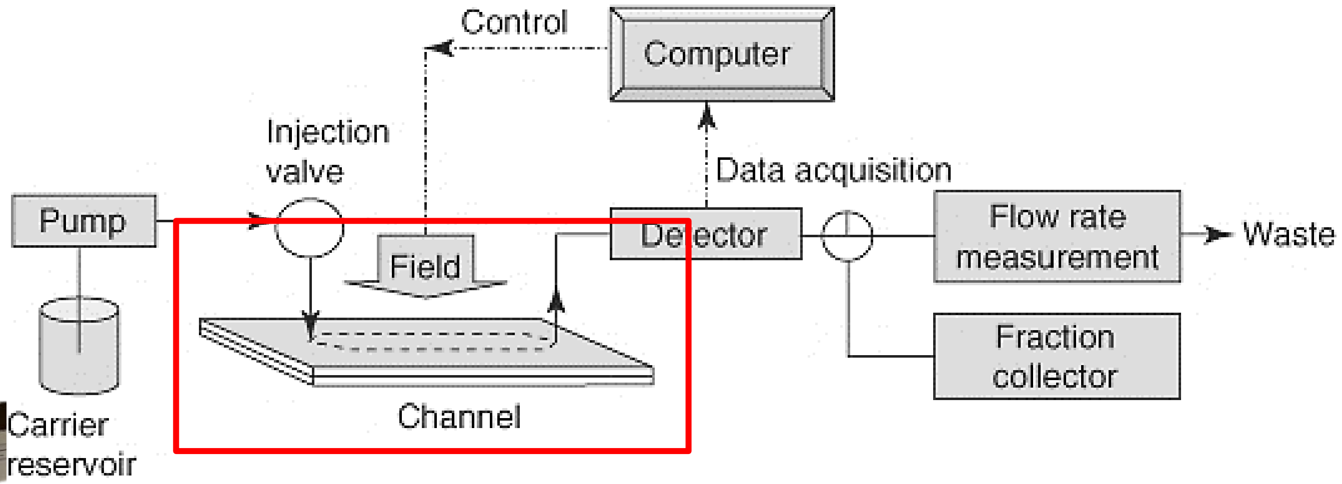


*Different "**Fields**" give rise to different FFF separation mechanisms based on a force interacting with an analyte's physicochemical properties:*



Williams, S. K. R., Runyon, J. R., Ashames, A. A. "Field-flow fractionation: addressing the nano challenge." *Anal. Chem.* **2011**, 83, 634–42.

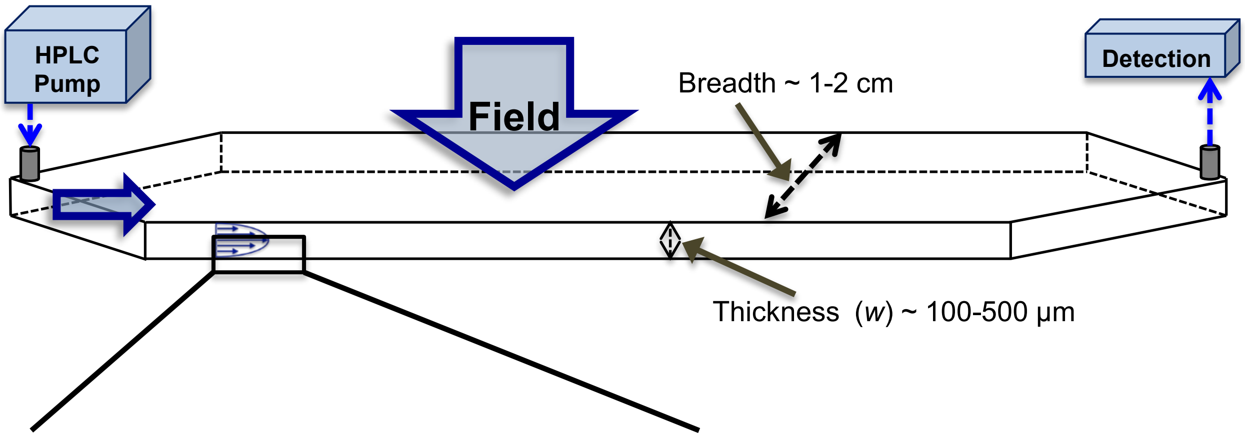
FFF System Assembled with Ancillary Equipment

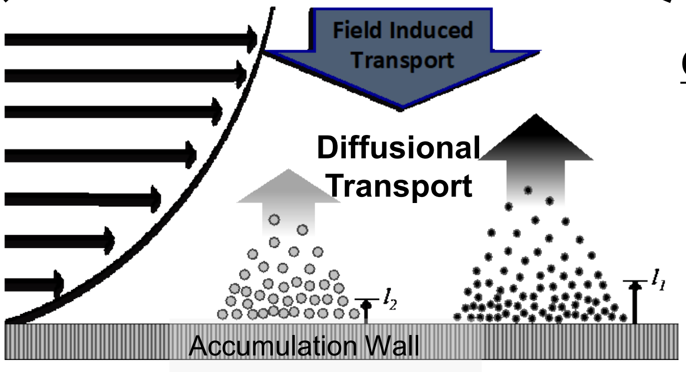


<u>Detection Options:</u>

Concentration Detection	UV, dRI, ELSD
Molecular Weight Determination	MALS, Viscometry
Size Determination	DLS (R_h), MALS (R_g)
Composition Sensitivity	UV, FL, ICP, MS

Field-Flow Fractionation Separation Basics

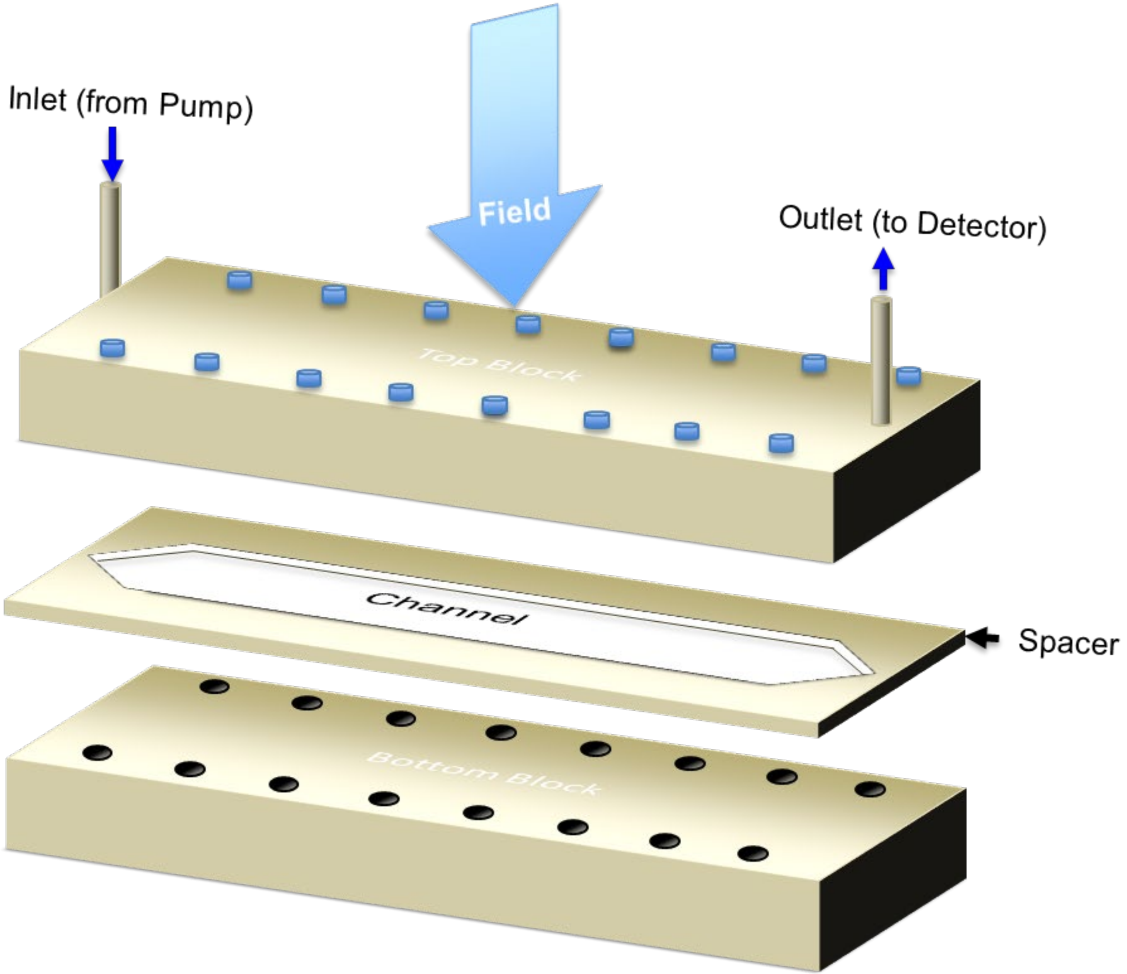




Origins of Retention

$$\frac{t_r}{t^0} = \frac{w}{6\ell} = \frac{|F|w}{6kT}$$

Advantages of FFF Open Channel



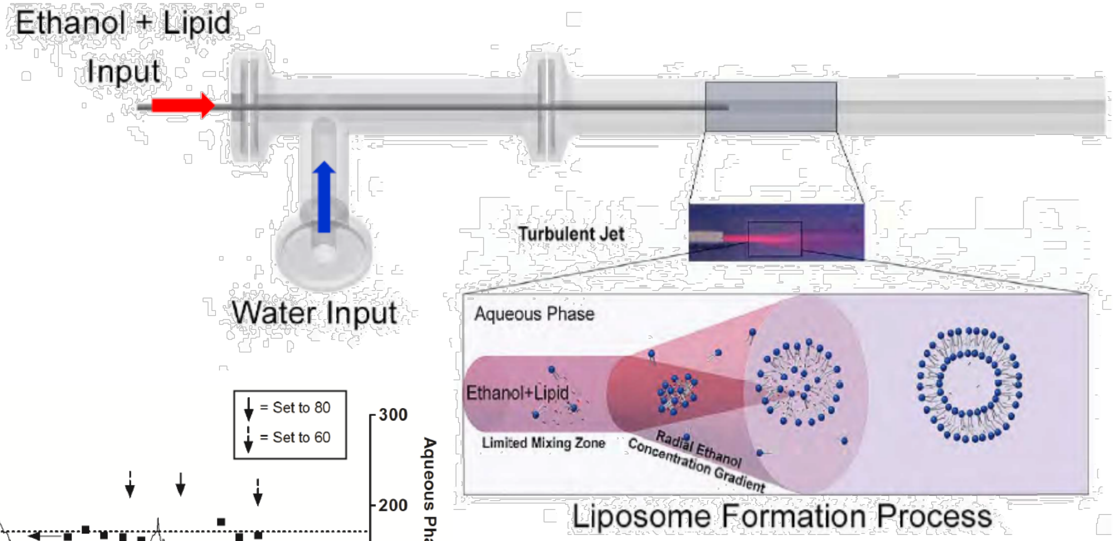
Design Benefits

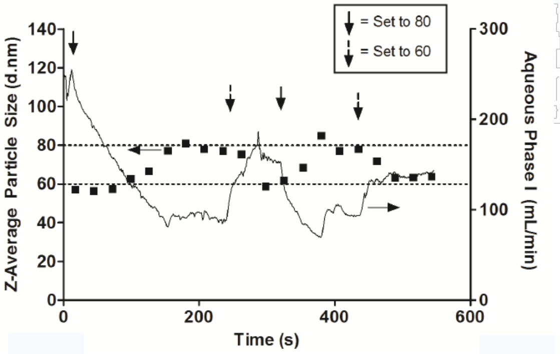
- Low shear stress
- Minimal sample prep
- Wide analyte size range
  - (0.001 - 70 µm)
- Choice of carrier fluids
- Limited sample loss
- ng to mg Quantities

Assessing morphological variability in liposomal drug products using field-flow fractionation

UCONN Liposome CM Platform

Image Credit: A. Costa, UConn

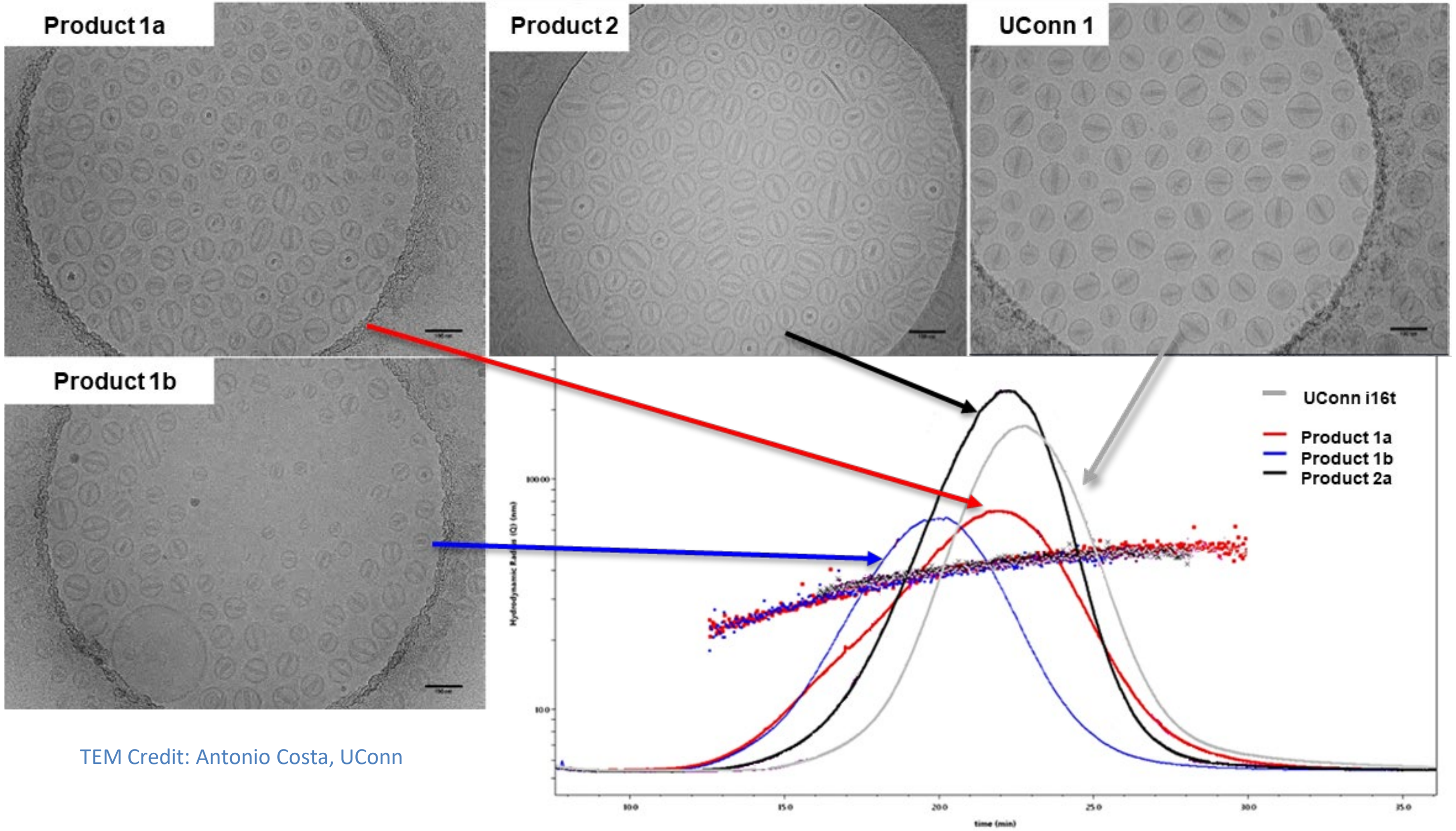




➢ **Control of particle size distribution, drug loading, and shape**

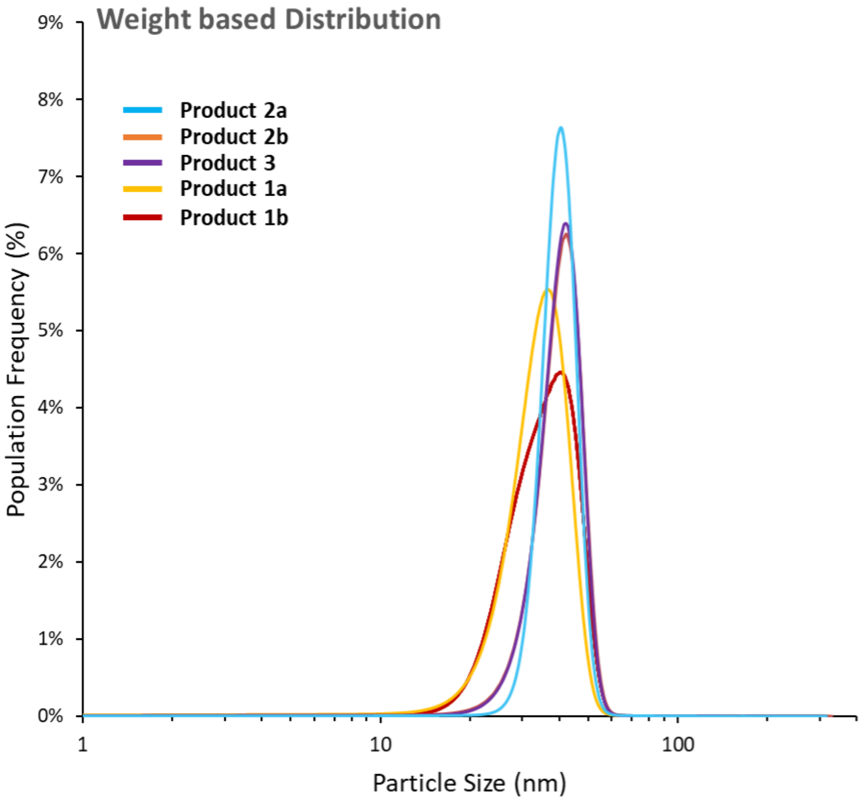
A. Costa, et al. Liposome formation using a coaxial turbulent jet in co-flow. Pharm Res. 2016, 33(4):404-416

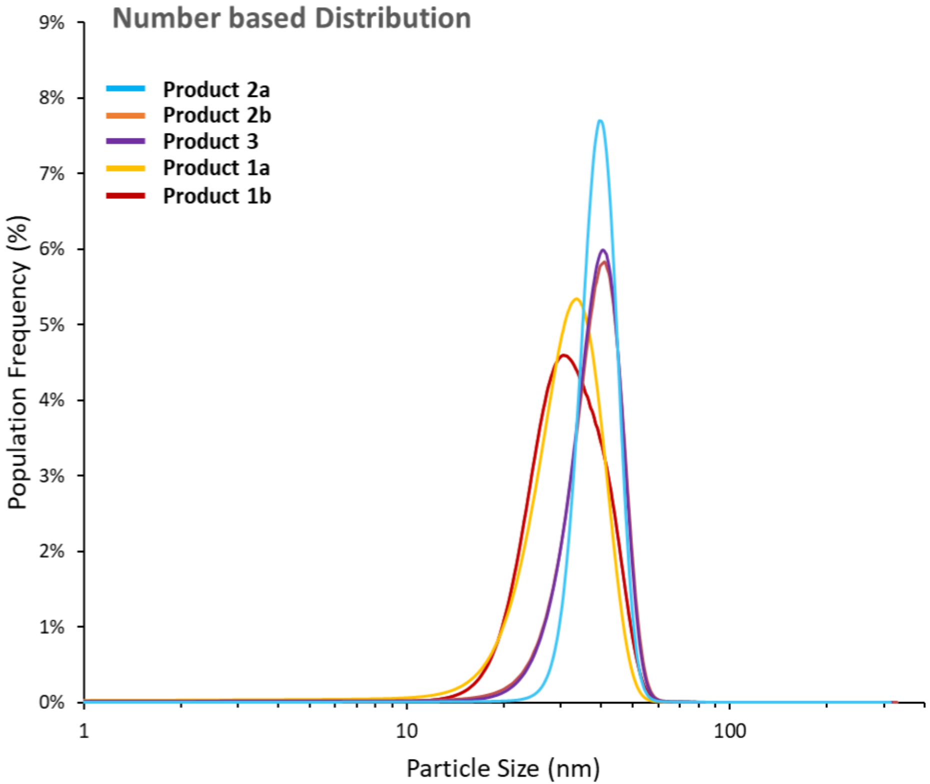
Doxorubicin Liposome Polydispersity



TEM Credit: Antonio Costa, UConn

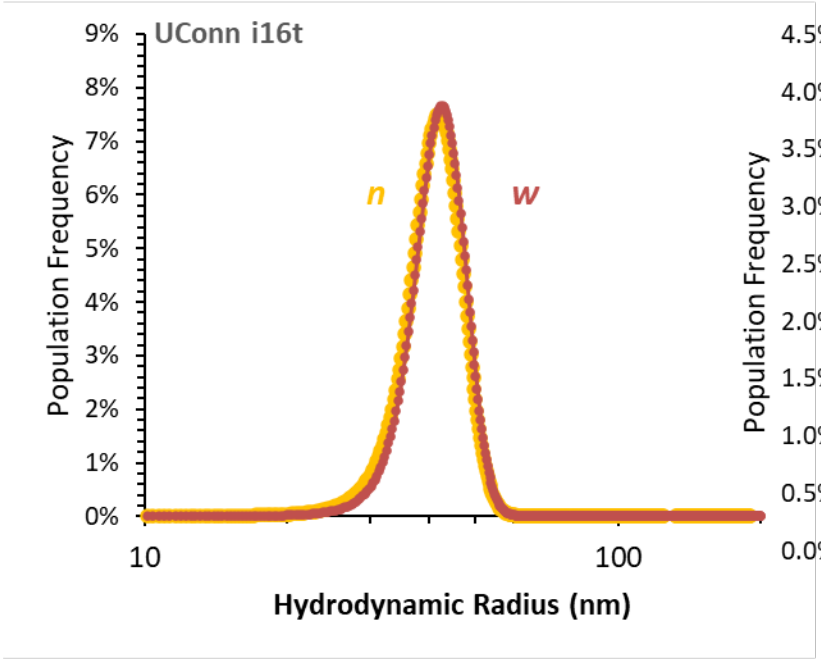
Determining Liposomal Particle Size Distribution

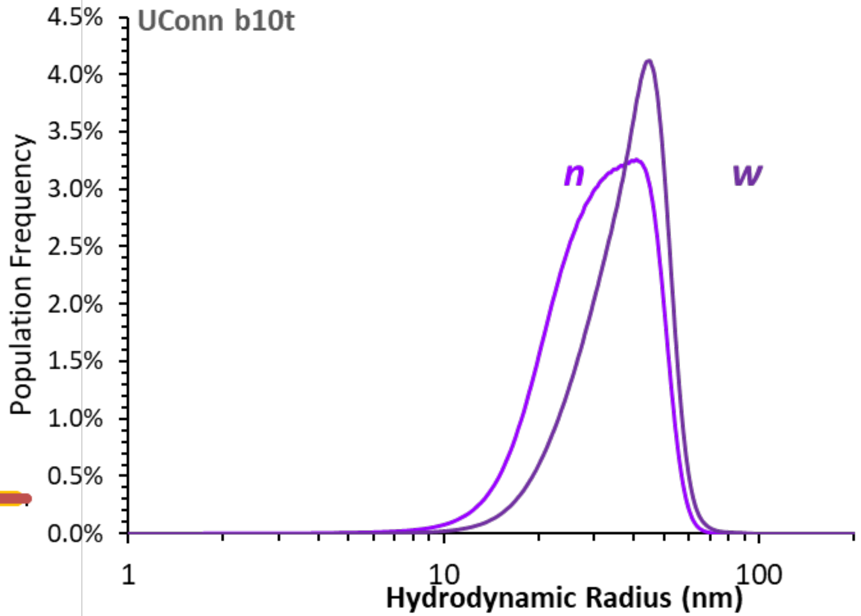




**Particle Size as hydrodynamic radius (R_h) from online DLS*

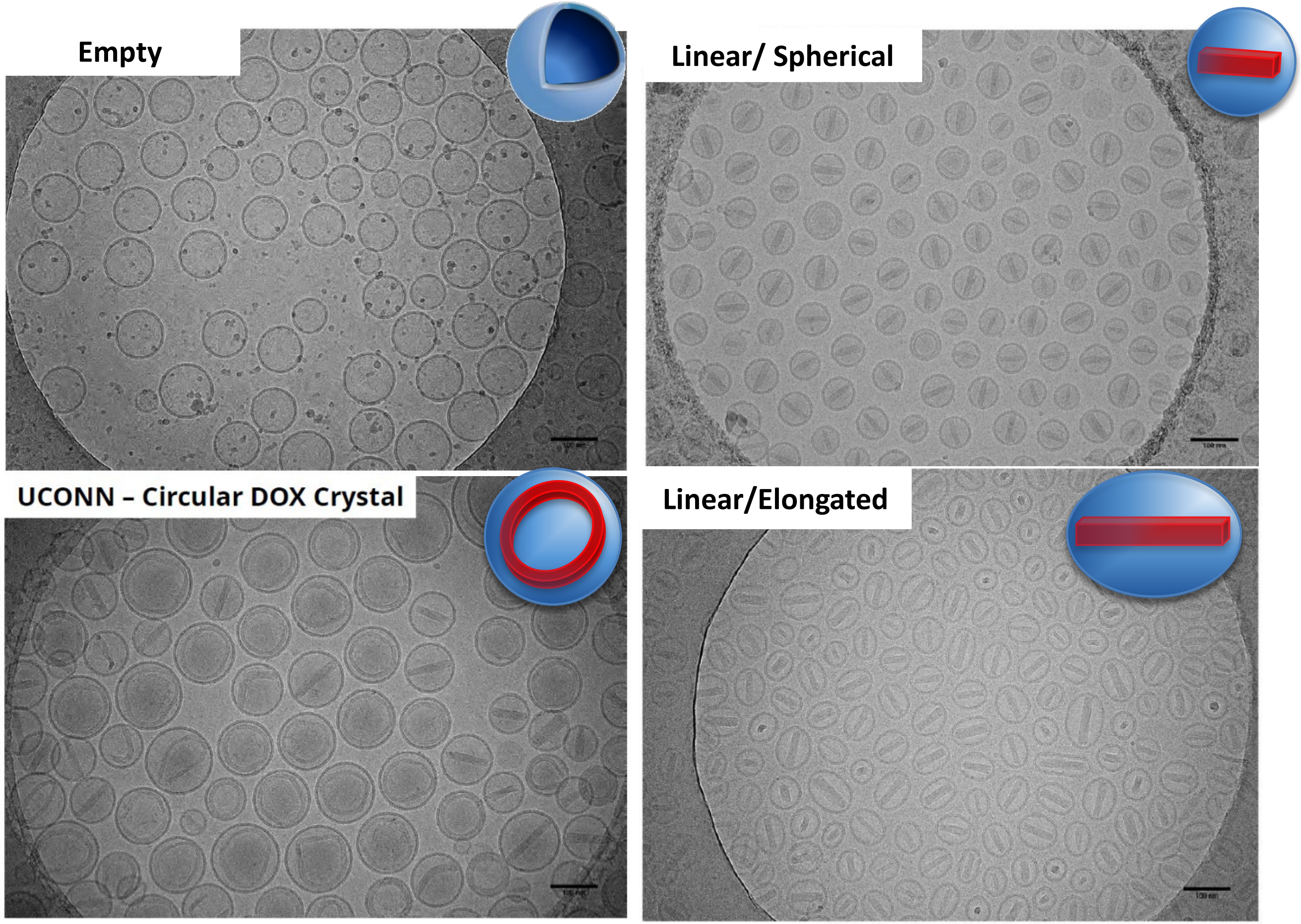
Determining Liposomal Particle Size Distribution

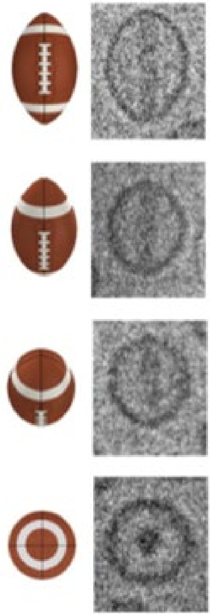




ID	Zavg (r.nm)	PDI (DLS)	rh(Q)n (nm)	rh(Q)w (nm)	PDI_n	PDI_w
b10t	43.4	0.061	36.8	45.7	0.101	0.064
i16t	43.7	0.017	41.4	42.9	0.016	0.015

Controlled Doxorubicin Liposome Morphology

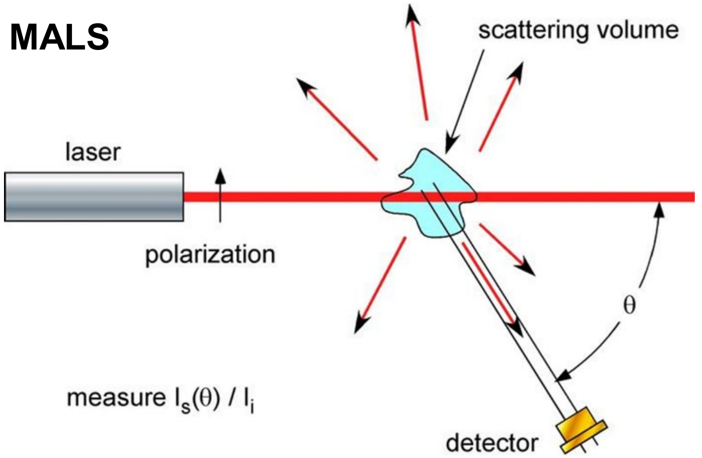




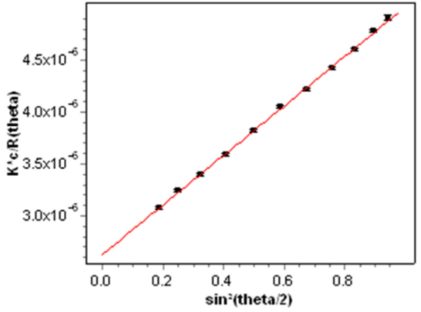
TEM Credit: Antonio Costa, UConn
Jiwen Zhang, FDA/CDRH

Light Scattering for Particle Size and Shape

MALS



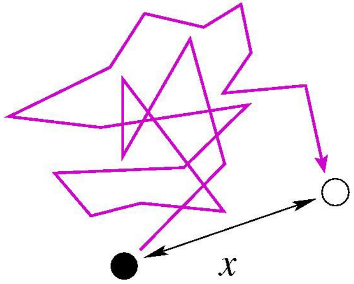
measure $I_s(\theta) / I_i$



$$\frac{K^*c}{R(\theta)} = \frac{1}{MP(\theta)} + 2A_2c$$

$$P(\theta) = 1 - \frac{16\pi^2 n_0^2}{3\lambda_0^2}\sin^2\left(\frac{\theta}{2}\right)\boxed{\left\langle r_g^2 \right\rangle} + \ldots$$

DLS



Stokes - Einstein Relation

$$D_t = \frac{kT}{6\pi\eta \boxed{R_h}}$$

		"true" r_{rms} of sphere							
		25		50		100		150	
		% relative error in measured quantity							
method	order of polynome fit	M_m	$r_{rms,m}$	M_m	$r_{rms,m}$	M_m	$r_{rms,m}$	M_m	$r_{rms,m}$
Debye	1	0.0	−1.7	−0.6	−4.8	−7.0	−21.1	−8.6	−21.8
	2	0.0	0.0	0.0	−0.3	−0.7	−3.5	−0.9	−3.9
	3	0.0	0.0	0.0	0.0	0.0	−0.4	−0.1	−0.4
Zimm	1	0.1	2.4	1.0	10.7	44.1	86.6	68.3	108.0
	2	0.0	0.0	0.0	−1.0	−5.7	−38.1	−7.8	−47.6
	3	0.0	0.0	0.0	0.1	1.0	8.1	1.5	10.6
Berry	1	0.0	1.4	0.5	5.7	13.8	31.3	18.5	34.9
	2	0.0	0.0	0.0	−0.3	−1.5	−8.6	−2.1	−10.2
	3	0.0	0.0	0.0	0.0	0.2	1.5	0.3	1.9

[a] The scattering species is assumed to be a compact sphere. Extrapolation based on 16 points in the angular interval 14−163°

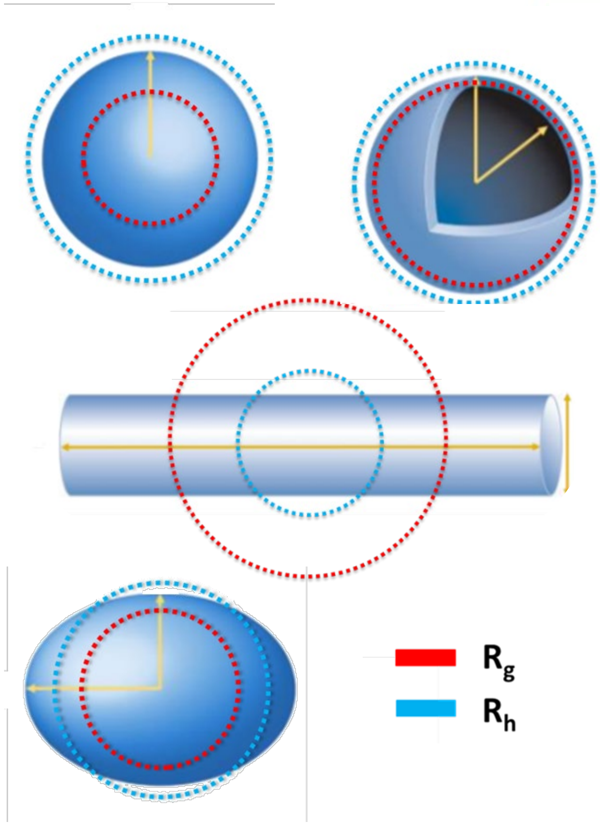
Figures brought to you by Wyatt Technology!

Andersson, M., et al., Analytical Chemistry 75.16 (2003): 4279-4291. DOI: 10.1021/ac030128+

Shape Factor (ρ) from Light Scattering

Table 1. Some representative shape factor values

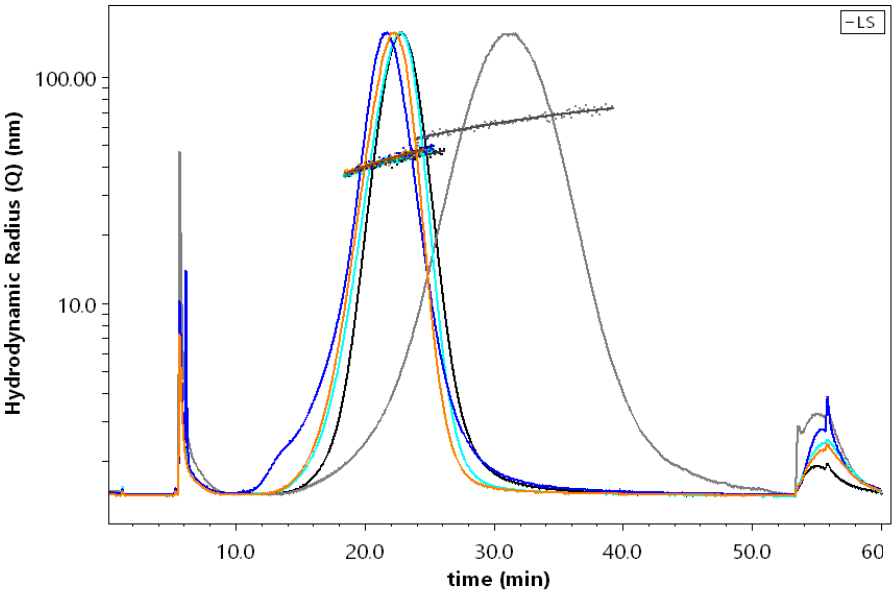
Structure	R_g	R_h	Shape factor
Uniform sphere with radius *R*	$R\sqrt{\frac{3}{5}}$	*R*	0.77
Hollow sphere with radius *R*	*R*	*R*	1
Spherical shell, p = ratio of inner radius r_i to outer radius *R*	$R\sqrt{\frac{3}{5}}\sqrt{\frac{1-p^5}{1-p^3}}$ [20]	*R*	$p = 0.5 \rightarrow \rho = 0.82$ $p = 0.9 \rightarrow \rho = 0.95$
Uniform rod, p = length / diameter = *L*/*d*	$\frac{L}{2}\sqrt{\frac{1}{3}+\frac{1}{2p^2}}$ [21]	$\frac{L/2}{\ln(p)+0.312+\frac{0.565}{p}-\frac{0.1}{p^2}}$ [21]	$p = 2 \rightarrow \rho = 0.85$ $p = 10 \rightarrow \rho = 1.55$
Uniform prolate ellipsoid, p = axial ratio *b*:*a*	$b\sqrt{\frac{1+2/p^2}{5}}$ [22]	$\frac{b\sqrt{1-\frac{1}{p^2}}}{\ln\left(p+\sqrt{p^2-1}\right)}$ [23]	$p = 2 \rightarrow \rho = 0.83$ $p = 10 \rightarrow \rho = 1.36$

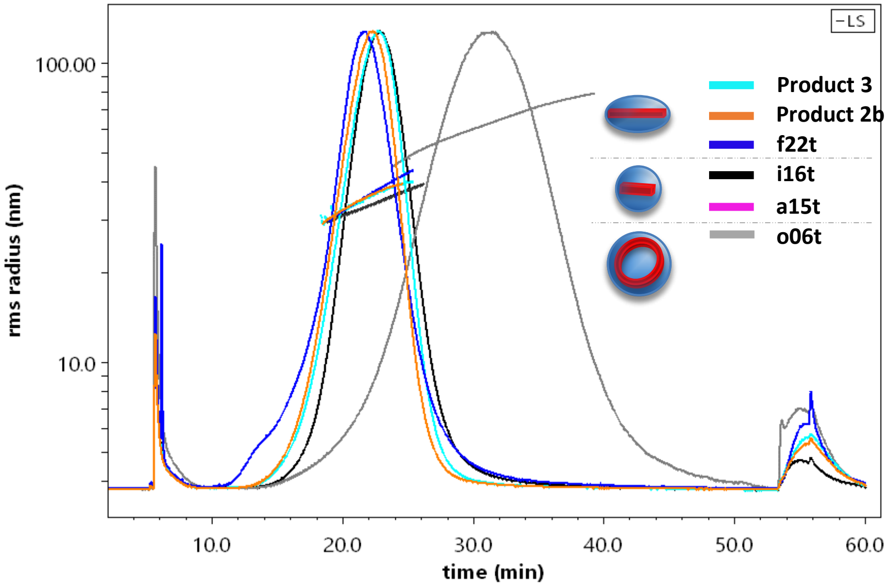


Wyatt White Paper: WP2611-Characterization-of-nano-pharmaceuticals-with-FFF-MALS-DLS

Doxorubicin Liposomes R_h and R_g

LS Model: Berry 2nd Order

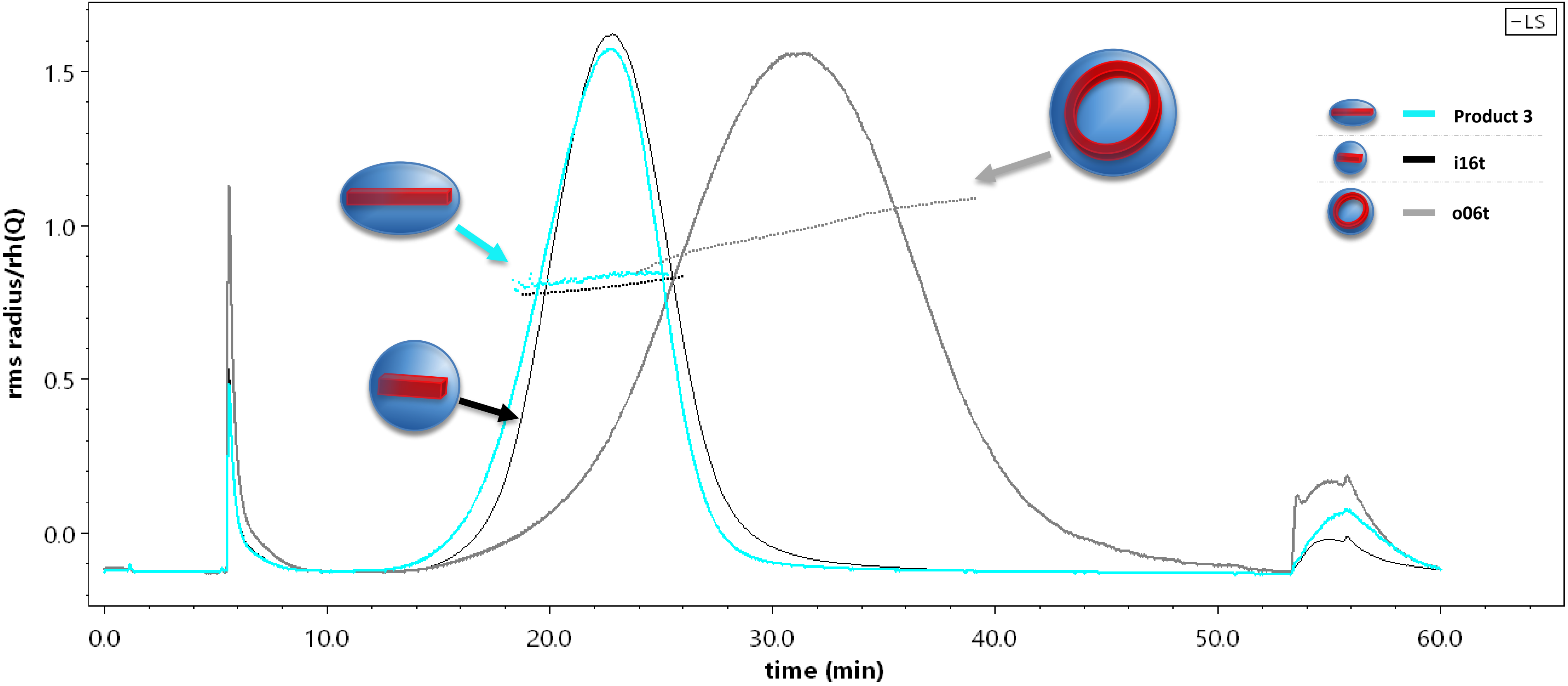




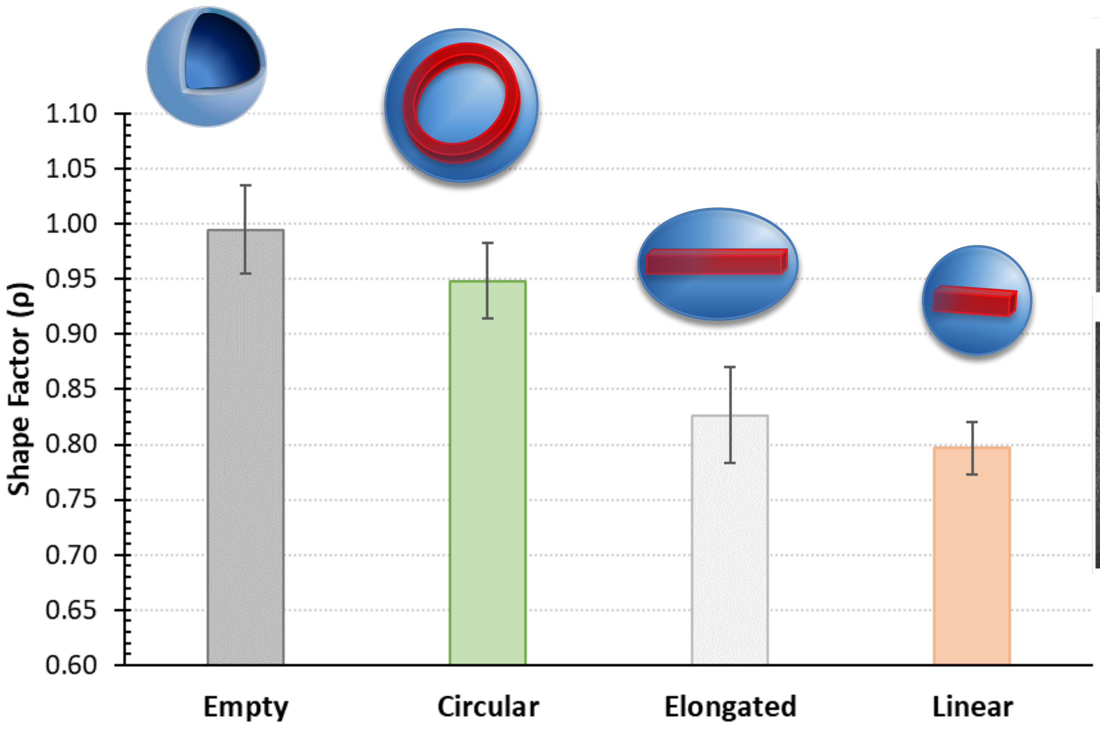
➢ **Slight variation in R_g values at equivalent retention times for elongated/spherical mixtures**

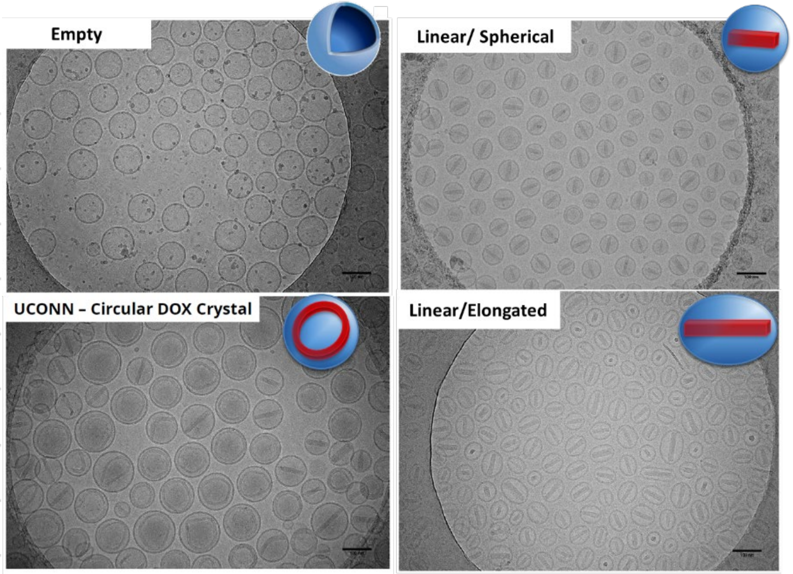
Doxorubicin Liposomes Shape Factors

LS Model: Berry 2nd Order



Doxorubicin Liposomes Shape Factors





	Shape Factor
Empty	$0.99_5 \pm 0.03$
*Elongated**	$0.83_4 \pm 0.04$
Circular	$0.94_8 \pm 0.03$
Linear	$0.79_7 \pm 0.02$

➢ **When is elongation elongated enough?**

Liposomal Analysis Take-Aways

- Continuous manufacturing provides the ability to produce liposomal samples of ***controlled*** size, shape, and polydispersity

- Future directions: Orthogonal methodologies for fractionated samples to assess morphological polydispersity: RMM, Cryo-TEM, etc.

- Further studies are essential to evaluate appropriateness of shape factor analysis as a rapid screening method
  - Comparisons of LS Models

Field-Flow Fractionation Protocols and Methods

FDA

NCL
Nanotechnology
Characterization
Laboratory

NCL Method PCC-19

Asymmetric-Flow Field-Flow Fractionation

Nanotechnology Characterization Laboratory
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
Frederick, MD 21702
(301) 846-6939
ncl@mail.nih.gov
https://ncl.cancer.gov

https://ncl.cancer.gov

Analysis of Nano-Objects using Field Flow Fractionation

Interlaboratory Comparison Protocol – Asymmetrical-Flow Field Flow Fractionation

This document contains information necessary to perform measurements and report results as a participant in the interlaboratory comparison for ISO Technical Specification 21362. It is designed to generate data necessary to establish baseline precision and reproducibility for asymmetrical-flow field flow fractionation with multiple detectors. This study is conducted under the auspices of VAMAS Technical Working Area 34.

EUNCL-PCC-022

EUNCL

FFF-MALS method development and measurements of size and molecular weight

Measurement of particle size distribution of protein binding, of mean molecular weight of polymeric NP components, study of batch to batch reproducibility, and study of release of free coating from NP surface by FFF-MALS

Standardization Efforts for Nanomaterial Analysis

Resources

- FFF: Addressing the Nano-Challenge
- Asymmetric Flow Field Flow Fractionation for the Characterization of Globule Size Distribution in Complex Formulations: A Cyclosporine Ophthalmic Emulsion Case
- Nanoparticle Manufacturing – Heterogeneity through Processes to Products
- Liposome formation using a coaxial turbulent jet in co-flow
- Orthogonal and complementary measurements of properties of drug products containing nanomaterials
- Physical characterization of liposomal drug formulations using multipdetector asysmetrical-flow field flow fractionation
- Improved multidetector asymmetrical –flow field flow fractionation method for particle sizing and concentration measurements of lipid-based nanocarriers for RNA delivery
- ISO TS21362 Nanotechnologies — Analysis of nano-objects using asymmetrical-flow and centrifugal field-flow fractionation
- NCL Method PCC-19: Asymmetric-Flow Field-Flow Fractionation
- EUNCL-PCC-022 FFF-MALS method development and measurement of size and molecular weight
- ASTM E3323-22: Standard Test Method for Lipid Quantitation in Liposomal Formulations Using High Performance Liquid Chromatography (HPLC) with an Evaporative Light-Scattering Detector (ELSD)
- ASTM E3324-22: Standard Test Method for Lipid Quantitation in Liposomal Formulations Using Ultra-High-Performance Liquid Chromatography (UHPLC) with Triple Quadrupole Mass Spectrometry (TQMS)
- ASTM E3297-21: Standard Test Method for Lipid Quantitation in Liposomal Formulations Using High Performance Liquid Chromatography (HPLC) with a Charged Aerosol Detector (CAD)

Acknowledgements

FDA Collaborators:

Jiwen Zheng

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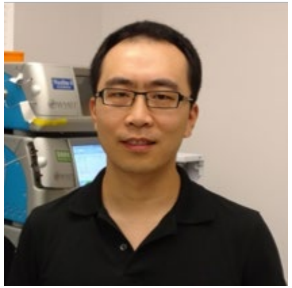
Goutam Pauli

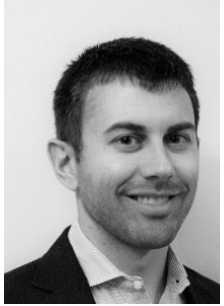


Haiou Qu

Xiaoming Xu

UCONN SCHOOL OF PHARMACY

Dr. Antonio Costa



Dr. Diane Burgess

Dr. Gowtham Yenduri

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- Critical Path
- Regulatory Science Research
- NanoCORES
- GDUFA

Questions?

William C. Smith, Ph.D.
Research Scientist
Division of Product Quality Research, Office of Testing and Research
CDER | US FDA