High Sensitivity Tools for Vaccine Development and Quality

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Hosted by Julie Nguyen and HORIBA Scientific

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Kevin Dahl, PhD - 20 years of particle and spectroscopic experience in pharma

- Technology Consulting
  - Instrumentation, laboratory
- Data Consulting
  - Reanalysis, interpretation, CMC support
- Document Consulting
  - Drafting and Review
- Method Consulting
  - Development, Optimization, Qualification, Validation
- Experimental Consulting and Services
  - Study design, execution, analysis, interpretation
- Training Services
  - Selected Instruments

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- ***The reward for work well done is the opportunity to do more.***

- Jonas Salk

Challenges in Vaccine Development

- Discovery
  - Rapid viral evolution – antigenic shift
- Formulation
  - Stabilization/delivery
- Storage and transport
  - Freeze-thaw
- Stability (ICH Q5C)
  - Toxic/immunogenic species
- In-use stability
  - Thaw/recon -> patient
- Release testing
  - Quality (ICH Q6B/Q6A)

Particulate and Spectroscopic Analytical Tools

Regulatory

- Modern vaccines inhabit a gray area between biotherapeutics (DS) and small molecule suspensions (DP)
  - Drug substance covered by ICH Q5A(R1) (safety)
  - Above deal with viral contamination/clearance, concentration, etc.

- What about drug product characteristics as CQAs?

- WHO Expert Committee on Biological Standardization, DRAFT
  Evaluation of the quality, safety and efficacy of messenger RNA vaccines for the prevention of infectious diseases: regulatory considerations, 2021

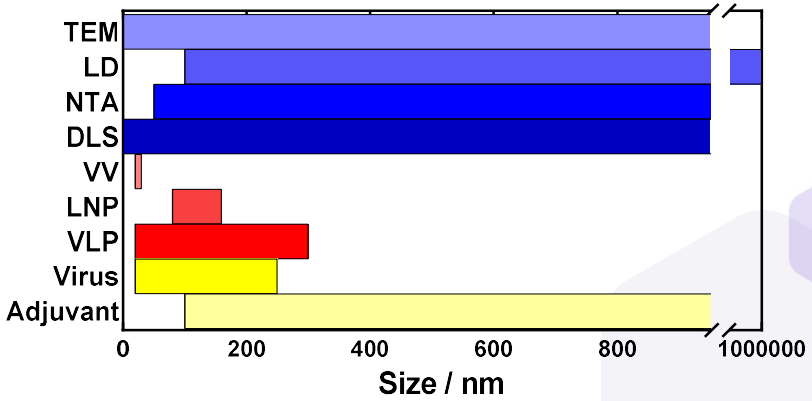
Particle size distribution (purity, consistency, safety)	light scattering such as dynamic or static light scattering; nanoparticle tracking analysis; electron microscopy; size-exclusion chromatography

Vaccines and Analytics

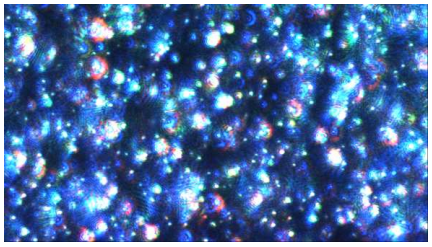
- Vaccine Delivery
  - Live Attenuated or Inactivated Virus - Polio
  - Adjuvanted - Nuvaxovid
  - Lipid Nanoparticle (LNP) – Pfizer/Moderna
  - Viral Vector/AAV – J&J
  - Virus Like Particle (VLP) – Gardasil

PARTICLES!

- Relevant Size Ranges and Common Techniques
  - Cryo-TEM
  - DLS
  - NTA
  - Laser Diffraction (LD)



Nanoparticle Tracking



- Based on video taken of point-scattering from particles
  - Particle movement in suspension described by Brownian motion:

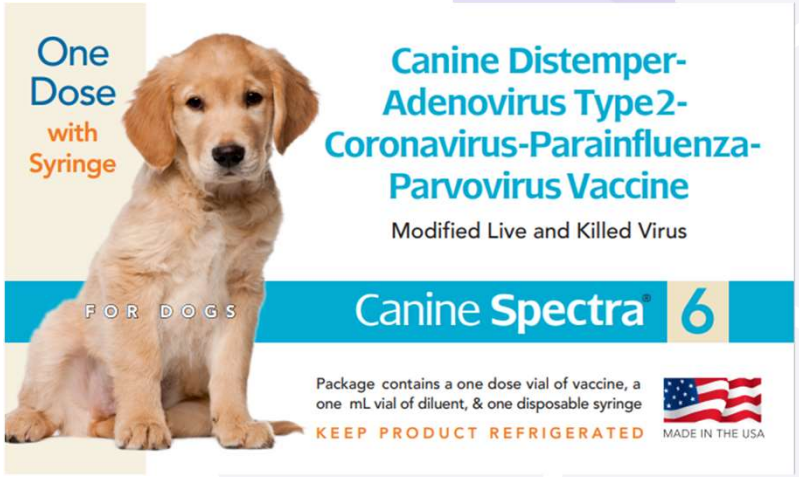
$$D_h = \frac{k_B \boldsymbol{T}}{3\pi \boldsymbol{\eta} D_m}$$

  - Light scattering techniques measure hydrodynamic radius (D_h)

- Strengths
  - Polydisperse (aggregated) samples
  - Single-particle (number-based) technique
  - Less sensitive to oversized material (relative to DLS)
  - Governed by ISO 19430, ASTM E2834-12

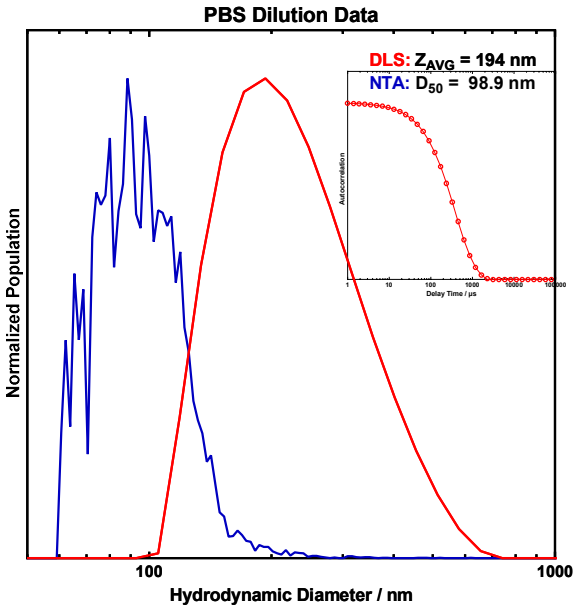
Experimental: Vaccine Analytics

- Lyophilized sample with included diluent (1 mL)
  - Modified live virus with adjuvant (proprietary), sub-Q delivery, pH 6-8
  - Reconstitution after aseptic diluent addition, “SHAKE WELL”
- Sample diluted 10^5 in PBS
  - Concentration range match for NTA instrument
- 3-laser NTA system (445/520/635 nm)
  - 350 µl sample stirred in cuvette
  - Practical limit of ~50 nm
  - Fluorescence capable



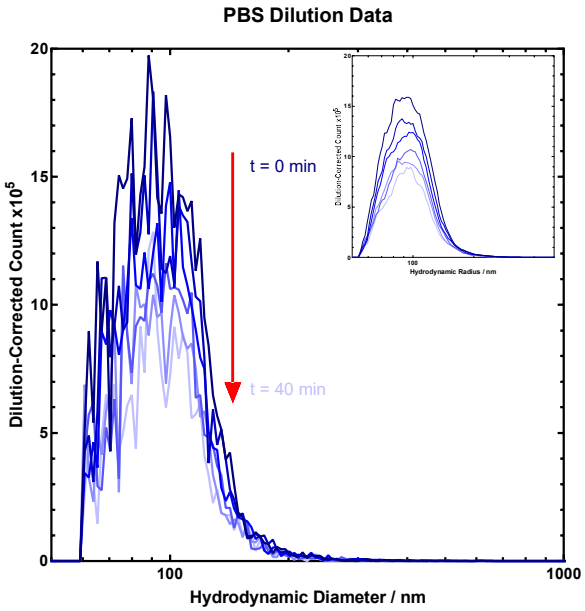
Reference: https://www.durvet.com/product/canine-spectra-6/

How things started:



- DLS results show a broad peak centered at 200 nm
  - Width suggests polydisperse sample – not optimal for DLS
  - Resolving power is ~x3/x5 for two modes
  - Intensity-based distribution – r^6 intensity dependence
- Number-based NTA shows a single mode at 100 nm
  - DLS signal dominated by a small amount of larger debris
    - Reconstitution/dilution?
  - NTA signal characterizes main population in sample

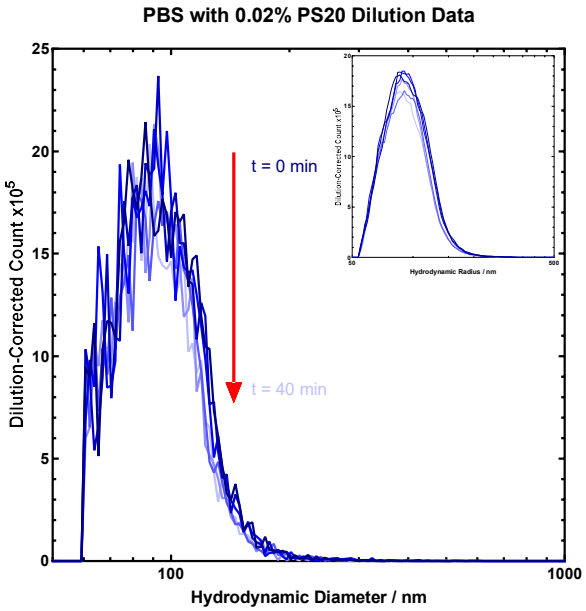
NTA Results: Dilution Only



- 40 min collection time for six replicates
  - 50% decrease in particle concentration after 40 min
    - Access to concentration is critical!
  - D90 of 140 nm (no prominent aggregation observed)
- Change in sample indicates instability
  - Dilution in PBS destabilizes the formulation

Sample	Rep	Counts	D50 (nm)	D90 (nm)	Conc. (part/mL)
Spectra 6 - PBS	1	5349	98.9	136.3	1.0E+13
Spectra 6 - PBS	2	4531	99.9	139.2	8.6E+12
Spectra 6 - PBS	3	4067	99.9	140.0	7.8E+12
Spectra 6 - PBS	4	3355	100.6	137.9	6.4E+12
Spectra 6 - PBS	5	3134	99.9	139.5	6.0E+12
Spectra 6 - PBS	6	2730	100.0	142.9	5.2E+12
	Average	**3861**	**99.9**	**139.3**	**7.4E+12**
	%RSD	**25.3**	**0.5**	**1.6**	**25.3**

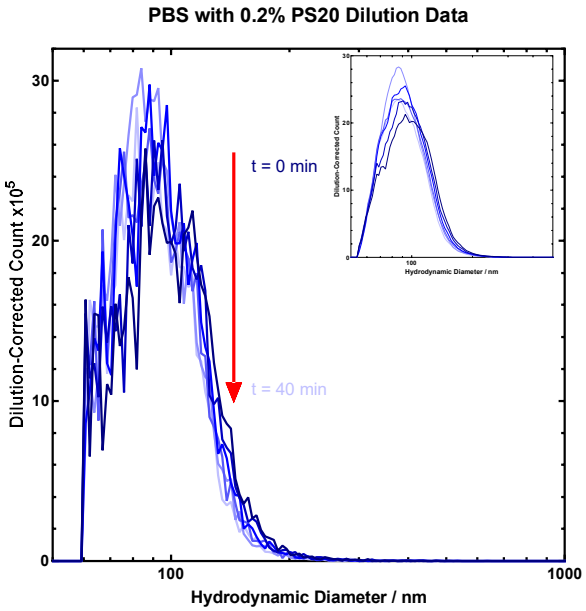
NTA Results: Dilution w/ 0.02% PS20



- Addition of 0.02% PS20 with PBS
  - PS20 should provide surface protection of unstable charge
- 40 min collection time for six replicates
  - ~15% decrease in particle concentration after 40 min
  - D90 has dropped to 132 nm

Sample	Rep	Counts	D50 (nm)	D90 (nm)	Conc. (part/mL)
Spectra 6 - PBS+0.02% PS20	1	5981	97.9	134.4	2.3E+13
Spectra 6 - PBS+0.02% PS20	2	5786	97.4	133.6	2.2E+13
Spectra 6 - PBS+0.02% PS20	3	5762	96.7	133.0	2.2E+13
Spectra 6 - PBS+0.02% PS20	4	5123	96.3	131.7	2.0E+13
Spectra 6 - PBS+0.02% PS20	5	5121	94.8	129.8	2.0E+13
Spectra 6 - PBS+0.02% PS20	6	5003	94.5	131.2	1.9E+13
	Average	**5463**	**96.3**	**132.3**	**2.1E+13**
	%RSD	**7.79**	**1.44**	**1.29**	**7.79**

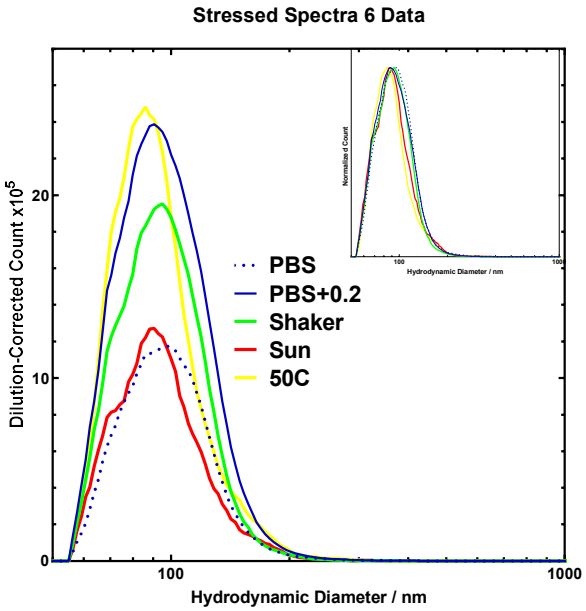
NTA Results: Dilution w/ 0.2% PS20



- Addition of 0.2% PS20 with PBS
- 40 min collection time for six replicates
  - No decrease in particle concentration after 40 min
  - D90 135 nm
  - PS20 reversing "hidden" aggregation?
    - Distribution width shifting and narrowing

Sample	Rep	Counts	D50 (nm)	D90 (nm)	Conc. (part/mL)
Spectra 6 - PBS+0.2% PS20	1	7665	104.1	143.9	2.9E+13
Spectra 6 - PBS+0.2% PS20	2	7852	100.6	140.6	3.0E+13
Spectra 6 - PBS+0.2% PS20	3	8009	97.2	135.2	3.1E+13
Spectra 6 - PBS+0.2% PS20	4	7599	96.0	133.5	2.9E+13
Spectra 6 - PBS+0.2% PS20	5	7904	93.8	129.8	3.0E+13
Spectra 6 - PBS+0.2% PS20	6	7100	94.4	129.6	2.7E+13
	Average	**7688**	**97.7**	**135.4**	**2.9E+13**
	%RSD	**4.24**	**4.07**	**4.28**	**4.24**

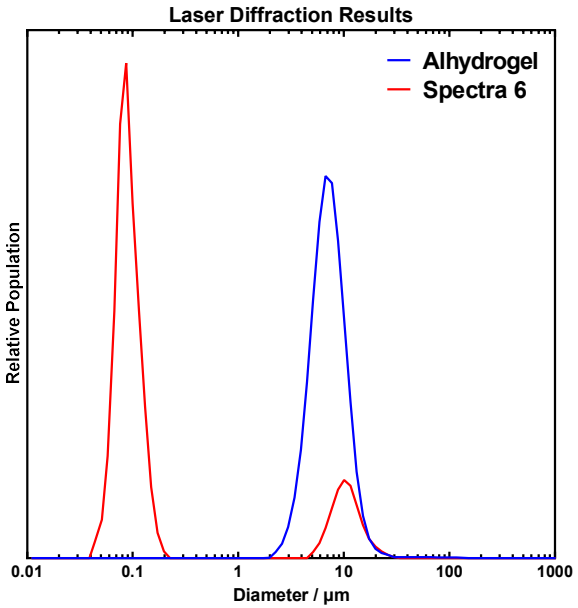
NTA Results: Additional Work



- Three stress conditions tested for 48 hours
  - Shaker plate, direct sunlight, 50 ℃
- Sample tolerates shaking
- Sample tolerates elevated temperature well
- Sample does not tolerate direct sunlight (UV)

Sample	Cond.	Counts	D50 (nm)	D90 (nm)	Conc. (part/mL)
Spectra 6 - PBS	None	3861	99.9	139.3	7.4E+12
Spectra 6 - PBS+0.2% PS20	None	7688	97.7	135.4	2.9E+13
Spectra 6 - PBS+0.2% PS20	48 hr. Shake	6693	98.9	133.6	2.6E+13
Spectra 6 - PBS+0.2% PS20	48 hr. Sun	3648	93.6	139.6	1.4E+13
Spectra 6 - PBS+0.2% PS20	50C	6828	91.2	137.4	2.6E+13

In Addition…



- Laser diffraction records scattering angle vs. intensity of scatter from suspension
  - Widely used for powder/high conc. suspensions
  - Minimum volume ~5 mL
  - Volume-weighted (r^3) distribution, can go below 100 nm
- LD is applicable to PS determination in vaccines
  - Products contain high concentration of particles
- Spectra 6 product shows band at 10 µm in addition to adjuvant band
  - Likely due to incomplete reconstitution
  - Explains poor DLS performance for same product!

Summary

- Vaccines are analytically ‘different’
  - Biologic product necessitates specific testing
  - Particles are the product, testing with new technologies
- Incorporation of particle techniques
  - Directly measure CQA of Drug Product
  - Useful in formdev, stability, transport, and in-use studies
  - Can be readily worked into product release workflow
- Kevin Dahl, PhD: kd@particlesellc.com