Assessing High-Resolution Nanoparticle Counting Techniques for the Regulatory Science of Complex Drug Products

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Purpose

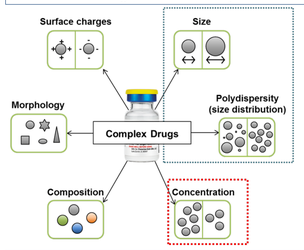
Purpose: Evaluating new *in vitro* measurements and standards to demonstrate pharmaceutical equivalence (Q1/Q2/Q3) and thereby ensure bioequivalence of complex generic products

Product similarity-Q1: Same excipient components, **Q2**: Same quantities, **Q3**: Physicochemical properties that are considered to be critical quality attributes of the drug product, as determined on a case-by-case basis

Common Q3 properties of complex formulations (e.g., emulsion, liposome, or colloids) that may be dependent on excipient quality and manufacturing conditions include: drug particle size distribution (PSD), morphology, pH, zeta potential, osmolality, rheological behavior, and *in vitro* drug release.

$$Concentration\ (C) = \frac{6 \times 10^{10} \times S \times \rho_L}{\pi \times \rho_s \times (particle\ diameter, d)^3}$$

Particle concentration may significantly affect formulation stability, delivered dose amount, and dissolution rate, thus impacting the safety and efficacy of these products. Therefore, measuring and understanding differences between product batches or brand-name and generics could be a useful tool in assessing product quality and bioequivalence.



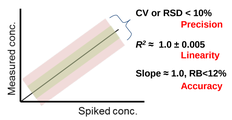
Herein we examine new analytical technologies for the **Q3 measurement of particle concentration** as a potential regulatory criteria of complex drugs. To achieve this we:

- Examined fundamental principles of new technologies
- Evaluated the accuracy, precision, and robustness using NIST size standards
- As a proof of concept we tested the methodology on approved generic propofol products

Is particle concentration an important quality parameter?

Introduction for Particle Counting Methods

Ensemble concentration: total mass/number per volume [g/mL]	Single particle counting methods: molar concentration [#/mL]
Gravimetric measurement	**Tunable Resistive Pulse Sensoring (TRPS)**
Dynamic Light Scattering (DLS)	**Nanoparticle Tracking Analysis (NTA)**
Turbidity (UV-Vis spectroscopy)	**Resonant Mass Measurement (RMM)**
Laser-induced breakdown detection	Imaging techniques using microscopy

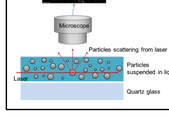


$$Relative\ Bias\ (RB)\% = 100 \times \left(\frac{measured}{theoretical} - 1\right)$$

Commercial particle analyzers capable of measuring nanoparticle concentration

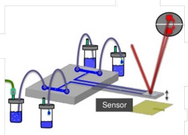
NTA

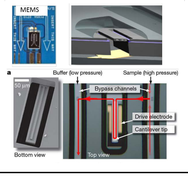
- Light scattering generated by a particle in suspension
- $D_t = \frac{2k_BT}{3\pi_h\pi\eta}$
- Conc. ≈ $\frac{\text{Number of Events}}{\text{Analytical Vol.}}$
- Analytical vol. ~4 nL (360 µm H, 360 µm W, 20 µm Depth of field, 10x magnification)



RMM

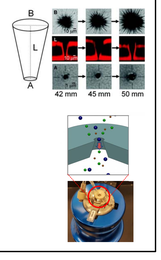
- Harmonic frequency of a micrro-resonator
- $D = \sqrt[3]{\frac{6M}{\pi\rho_{particle}}}$
- Conc. ≈ $\frac{\text{Number of Events}}{\text{Analytical Vol.}}$





TRPS

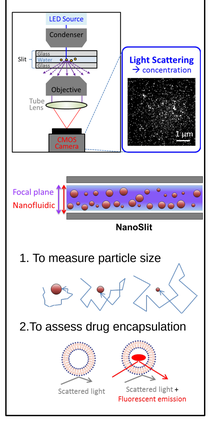
- Ionic resistance generated by a particle inside a pore
- Count rate $J = C \times$ flow rate Q
- Test C_2 = Stand $C_1 \times \left(\frac{\text{Sample Freq.}}{\text{Stand Freq.}}\right)$



NIST developed device

Nanofluidic Slit with Tracking

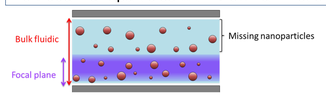
- Light scattering generated by a particle in suspension
- $D_t = \frac{2k_BT}{3\pi_h\pi\eta}$
- Conc. ≈ $\frac{\text{Number of Events}}{\text{Analytical Vol.}}$



Experimental Methods

Instrument 1: Nanoparticle Tracking Analysis (NTA)

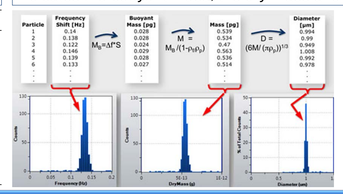
NTA is based on the Stokes-Einstein equation for the diffusion coefficient of individual nanoparticles with Brownian motion and light scattering monitoring.

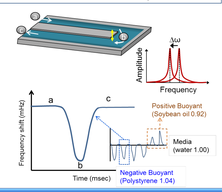


	ZetaView (Particle Metrix)
Camera	sCMOS (640 x 480 pixels)
Lasers (automatic selection depending on the applications)	14 mW at 375 nm; **>30 mW at 405 nm;** >30 mW at 488 nm; >30 mW at 520 nm; >30 mW at 660 nm
Magnification	X10 objective (20 µm depth of field, 703 nm/pixel, 90º laser scattering)
Focal plane #	1, 2, or 11 positions (multiple position scanning)
Sample vol. (mL)	~1 mL (5 mL) + peristaltic pumps

Instrument 2: Resonance Mass Measurement (RMM)

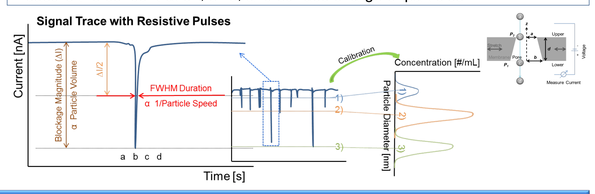
RMM is a technique to measure resonating frequency of the cantilever which indicates the buoyant mass, the dry mass and the size of the individual particle.





Instrument 3: Tunable Resistive Pulse Sensing (TRPS)

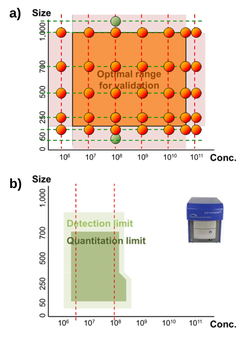
TRPS is based on the Coulter principle (electric resistive pulse sensing) to measure the concentration, size, and surface charge of particles.



Results

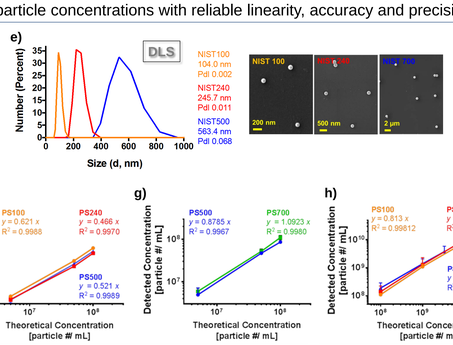
A. Range of Detection (size and concentration)

In screening a wide range of particle size and concentration (**a**), TRPS (**d**) showed the largest range of nanoparticle detection among three particle counting techniques including NTA (**b**) and RMM (**c**).



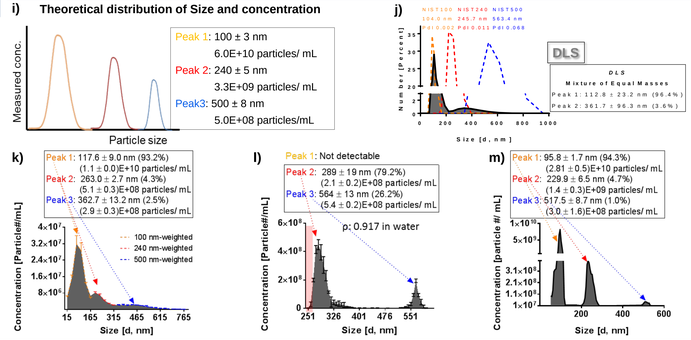
B. Linearity, Precision and Accuracy

DLS (**e**), NTA (**f**), RMM (**g**) and TRPS (**h**) showed the accurate size distribution from monomodal samples of PS size standards with three different sizes. Additionally, three particle counting techniques quantitatively measured the molar particle concentrations with reliable linearity, accuracy and precision.



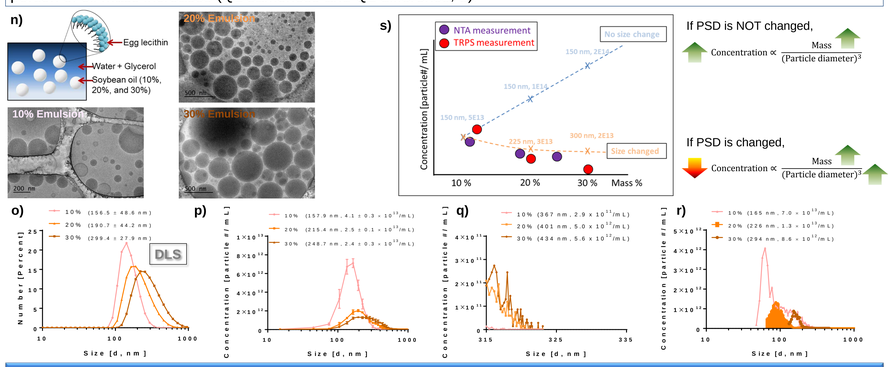
C. Assessment of Robustness

Heterogeneous samples were prepared by mixing of NIST100, NIST240 and NIST500 at equal mass (**i**). Compared to DLS (**j**), NTA (**k**) and RMM (**l**), TRPS showed the most accurate concentrations and size distribution and highest resolution of the heterogeneous mixture (**m**).



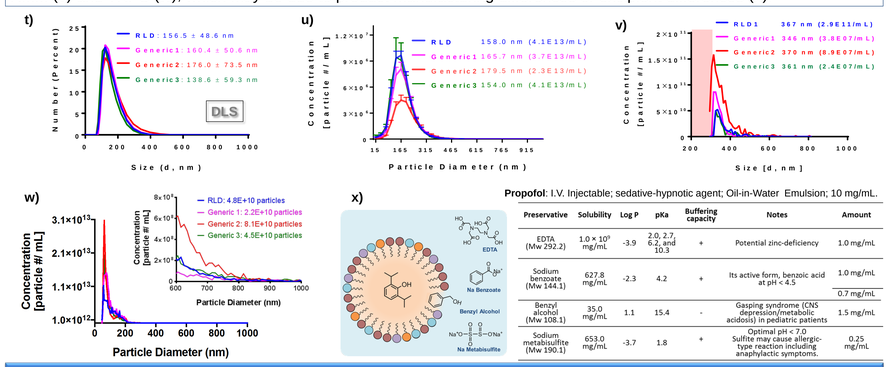
D. Assessment of Compatibility

A large majority of complex drug products are formulated as oil-in-water emulsions (**n**). Compared to RMM (**q**), DLS (**o**), NTA (**p**) and TRPS (**r**) were able to measure pharmaceutical variables, i.e., changed particle size and concentration, as the portion of oil mass increases (Q1 sameness and Q2 difference, **s**).



E. Drug Product Assessment (Propofol oil-in-water emulsion)

Size distribution (D50-SPAN) of DLS measurements is still within acceptable range in Population Bioequivalence (95% CI, **t**). However, more reference-to-generic differences in size and concentration distributions were observed using NTA (**u**), RMM (**v**) and TRPS (**w**), which may be due to product manufacturing differences or excipient differences (**x**).



Propofol: I.V. Injectable; sedative-hypnotic agent; Oil-in-Water Emulsion; 10 mg/mL.

Preservative	Solubility	Log P	pKa	Buffering capacity	Notes	Amount
EDTA (Mw 292.2)	1.0 × 10⁵ mg/mL	-3.9	2.0, 2.7, 6.2, and 10.3	+	Potential zinc-deficiency	1.0 mg/mL
Sodium benzoate (Mw 144.1)	627.8 mg/mL	-2.3	4.2	+	Its active form, benzoic acid at pH < 4.5	1.0 mg/mL 0.7 mg/mL
Benzyl alcohol (Mw 108.1)	35.0 mg/mL	1.1	15.4	-	Gasping syndrome (CNS depression/metabolic acidosis) in pediatric patients	1.5 mg/mL
Sodium metabisulfite (Mw 190.1)	653.0 mg/mL	-3.7	1.8	+	Optimal pH < 7.0 Sulfite may cause allergic-type reaction including anaphylactic symptoms.	0.25 mg/mL

Conclusions

Compared to the higher accuracy, resolution, and larger analytical range of TRPS, nanoparticle concentration measurements of NTA and RMM were more precise and reproducible. The size is still more sensitive measure of product difference, but the concentration measurement supports that. The observed reference-to-generic variations may be attributed to different manufacturing process or different excipients in the formulation, which will be further studied.

Future Plans

- Replicate analysis using more lots of propofol emulsion products.
- Fabrication and validation in-house nanofluidic slit device with better resolution and reproducibility.
- Characterization of other drug products containing nanomaterials, such as liposomes and emulsions.

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