New Tools to Improve Complex Drug Regulation

: Establishing simple and cost-efficient techniques for nanoparticle counting

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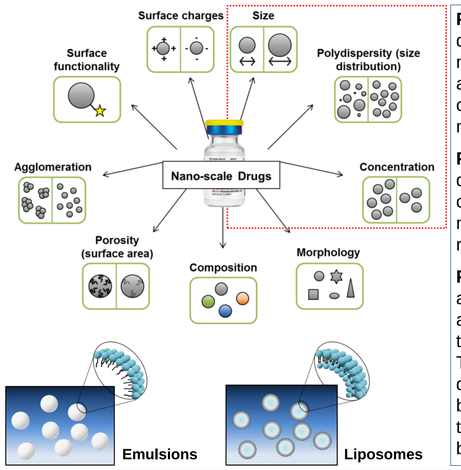
Purpose



Purpose: Investigating current and developing novel single nanoparticle measurement techniques to improve the assessment of physiochemical equivalence of complex pharmaceutical products including nanomaterials

Physicochemical properties of complex drug products (e.g. emulsion, liposome, or colloids): drug particle size distribution, morphology, pH, zeta potential, osmolality, rheological behavior, and *in vitro* drug release.

Particle concentration can 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 develop a novel, practical, and economical **nanoparticle concentration measurement technique** to characterize physiochemical properties of pharmaceutical complex products including nanomaterials. 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, tested the new technologies on a brand-name and three approved generic nanoemulsion products.

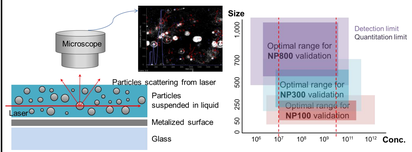
Introduction: Particle Concentration Methodologies

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

Commercial particle analyzers capable of measuring nanoparticle concentration

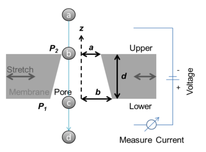
Nanoparticle Tracking Analysis (NTA)

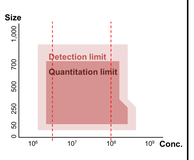
- Light scattering generated by a particle in suspension
- $D_t = \frac{2k_BT}{3\eta_h\pi\eta}$
- Conc. ≈ $\frac{\text{Number of Events}}{\text{Analytical Vol.}}$
- Analytical vol. (100 μm H, 80 μm W, 10 μm D)

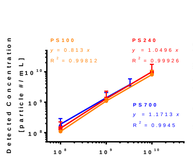


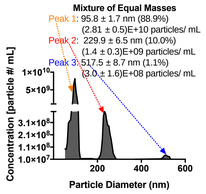
Tunable Resistive Pulse Sensing (TRPS)

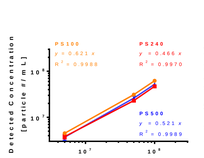
- Ionic resistance generated by a particle inside a pore
- Count rate $f = C \times$ flow rate $\boldsymbol{Q}$
- Test C_2 = Stand $C_1 \times \left(\frac{\text{Sample Freq.}}{\text{Stand Freq.}}\right)$

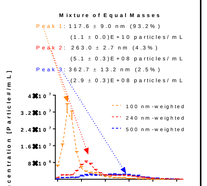












Why do we develop novel techniques with reliable accuracy and reproducibility?

	Advantages	**Disadvantages**
NTA	• Visualization of individual particles • Calibration-free • High reproducibility and precision • Automated system	• Inaccurate analytical volume (~5 nL) • Thick focal planes • Dependency on the camera setting • Narrow range of NP size and concentration
TRPS	• High-throughput accuracy • More accurate for polydispersed samples • Relatively large analytical volume (~2 μL) • Independent of NP composition	• Dependency on the accuracy of calibration • Sensitive to the changes of analytical conditions • Less reproducibility • Not fully automated

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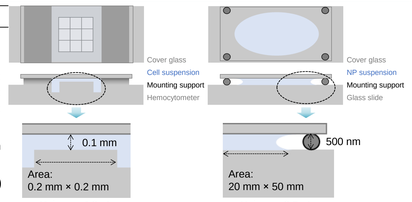
Background & Experimental

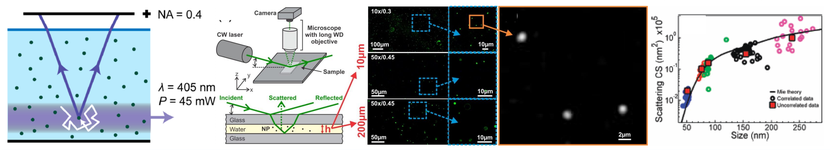
Method 1. Nanoslit

- To obtain a) independency on camera settings; b) well-defined analytical volume; and c) independency on the NTA programming.
- Near to single focal plane for improved accuracy, compatibility, and robustness.

	NTA 1	**NTA 2**
Camera	CCD or sCMOS	sCMOS
Lasers (automatic selection)	55 mW at 405 nm; 45 mW at 488 nm; 50 mW at 532 nm; 40 mW at 642 nm	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
Cell dimensions (H, W, D)	53 cm, 51 cm, 46 cm	25 cm, 20 cm, 30 cm
Magnification	X20 objective (173º laser scattering)	X10 objective (90º laser scattering)
Focal plane #	1 position	1, 2, or 11 positions
Sample vol. (mL)	~3 mL (10 mL)	~1 mL (5 mL)
Particle size	Hydrodynamic diameter	(Stokes-Einstein eq.)



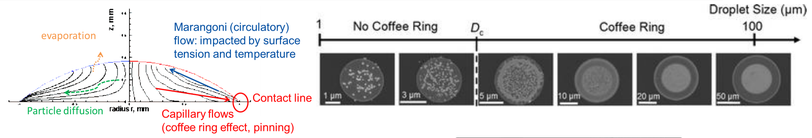
Ref.1 Edwin van der Pol, et. al., *Nano Lett.* **2014**, 14, 6195-6201.
Ref.2 Christoph Haiden, et. al., *Langmuir* **2014**, 30, 9607-9615.
Ref.3 A. Tcherniak, et. al., *Nano Lett.* **2010**, 10, 1398-1404.



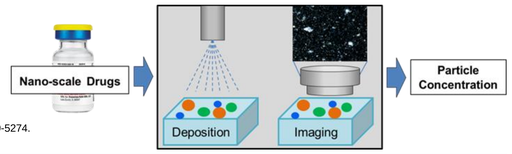
Method 2. Microdeposition

- To obtain a) independency on camera settings; b) well-defined analytical volume; and c) independency on the NTA programming.
- Mono-dispersion on smooth substrate for improved accuracy, compatibility, and robustness.

Sample (particle-to-particle interactions)	**Substrate (particle-to-substrate interface)**	**Evaporation (liquid-to-air interface)**	
Particle concentration	Roughness	Humidity	Control to low
Droplet size/volume	Impurity	Temperature	Control to high
Colloidal stability	Hydrophilicity	Media evaporation rate	Not controllable
Surfactant/salt	***Temperature (thermoconductivity)??***	Liquid heating by illumination while imaging	
Particle size/shape/density			



Ref.1 Hua Hu, et. al., *J. Phys. Chem. B* **2006**, 110(14), 7090-7094.
Ref.2 Xiaoying Shen, et. al., *J. Phys. Chem. B* **2010**, 114(16), 5269-5274.
Ref.3 Tak-Sing Wong, et. al., *Anal. Chem.* **2010**, 83, 1871-1873.



Results

Method 1. Nanoslit

Based on the calculated depth of field for all objectives, the nanoslit was fabricated to be 100, 500, or 700 nm in height, and the PS standards and nanoemulsion samples were characterized using a darkfield microscopy to obtain their molar concentrations in DDI water.

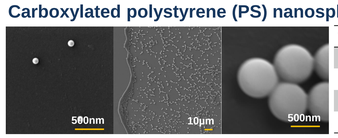
Depth of Field calculation

e **@ λ=550 nm**	**Plan Apochromat UW 10x (0.45NA)**	**Plan Apochromat UW 20x (0.75NA)**	**Plan Apochromat UW 50x (0.95NA)**	**Plan Fluor 100x (0.90NA)**
4 μm	3.60494 μm	1.24444 μm	0.63821 μm	0.72346 μm
14 μm	5.82716 μm	1.91111 μm	0.84874 μm	0.83457 μm
24 μm	8.04938 μm	2.57778 μm	1.05926 μm	0.94568 μm

$$d_{tot} = \frac{\lambda \cdot n}{NA^2} + \frac{n}{M \cdot NA} \times e$$

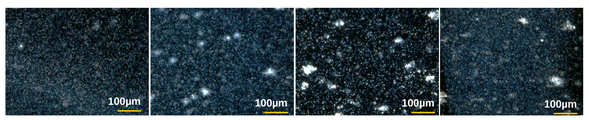
M: Lens-magnification;
NA: Objective numerical aperture;
λ: wavelength of light;
***n*: refractive index of medium-air (1.0) or oil (1.515);**
***e*: the smallest distance that can be resolved by a detector that is placed in the mage plane of the microscope objective, 4 – 24 microns)**

Carboxylated polystyrene (PS) nanospheres observed using SEM



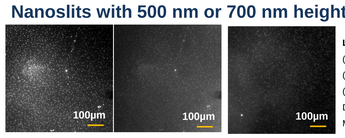
Thermo Nanospheres	**Size [nm]**	**Nominal Conc.**	**Measured Conc.**	
100 nm	3100A	100 ± 3	1.8×10^{13}/ mL	(a)150k
240 nm	3240A	240 ± 5	1.3×10^{12}/ mL	
500 nm	3500A	508 ± 8	1.5×10^{11}/ mL	
700 nm	3700A	707 ± 9	5.3×10^{10}/ mL	(b)150k,(c)2.1k

Nanoslits with less than 100 nm height



Legends (from left):
(1) RLD; (2) Generic 1; (3) Generic 2; and (4) Generic 3.
Dilution factor: 100
Dispersant: DDI water
Measured temperature: RT (~23°C)
20x objective

Nanoslits with 500 nm or 700 nm height



Legends (from left):
(1) PS 100 nm (~E8/mL, 20x objective, halogen lamp):
(2) PS 100 nm (~E8/mL, 20x objective, LED 625 nm);
(3) Generic 1 (~E11/mL, 1K dilution, 20x objective, halogen lamp).
Dispersant: DDI water
Measured temperature: RT (~23°C)

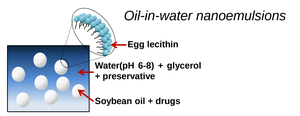
Advantages of Method 1
- Relatively less dilution and modification of the analytes
- Brownian motion-based size measurement

Disadvantages of Method 1
- Need to well-define the thickness
- Drying from the edge

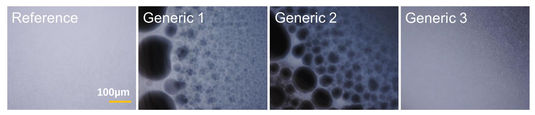
Method 2. Microdeposition

Collective effects of the liquid evaporation, capillary flow velocity, and the particle diffusion velocity within sessile droplet mediate another level of self-assembly or partial monolayer deposition of oil globules on the Si substrate.

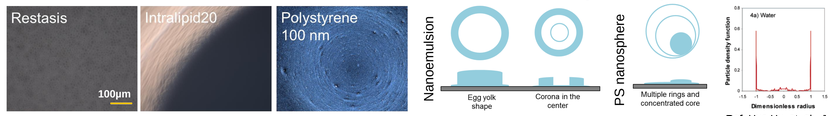
Oil-in-water Nanoemulsion



Coffee ring effect mediates another level of self-assembly.

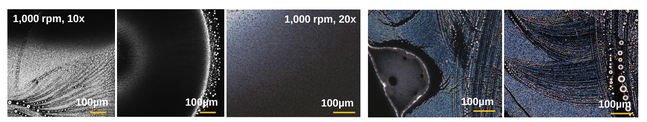


Other nanoemulsion and PS standards also show different microdeposition patterns.



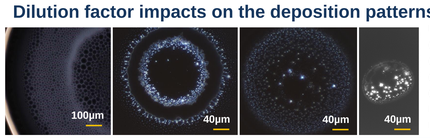
Ref. Hua Hu, et. al., *J. Phys. Chem. B* **2006**, 110(14), 7090-7094.

Partial monolayer of oil globules after agitation (centrifugal force or spreading)



Legends (from left):
(1-2) 1,000 rpm, 10x objective;
(3) 1,000 rpm, 20x objective;
(4-5) Manual spreading, 20x objective.
Dilution factor: 0 (no dilution, ~E14/ mL)
Measured temperature: RT (~23°C)

Dilution factor impacts on the deposition patterns.



Legends (from left):
(1) No dilution (~E14/ mL, 20x objective);
(2) 1K dilution (~E11/ mL, 50x objective);
(3) 1M dilution (~E8/ mL, 50x objective);
(4) 1B dilution (~E5/ mL, 50x objective).
Dispersant: DDI water
Measured temperature: RT (~23°C)

Advantages of Method 1
- No need to define the analytical volume
- Higher refractive index in air than in water
- Easy and simple

Disadvantages of Method 1
- Significant modification of the analytes
- Impacts of drying conditions

Conclusions

To simply and directly measure the nanoparticle concentration of complex nanoemulsion formulation without any other background information, nanoslit and microdeposition approaches were used to make nanoparticles in one focal plane, which was characterized and analyzed using optical darkfield microscopes. The measured concentration via the novel approaches was compatible to that obtained using other single nanoparticle counting techniques, TRPS and NTA.

Future Plans

- Validation of the novel approaches using NIST size standards
- Concentration measurement of the approved nanoemulsion products from different manufacturers
- Characterization of other drug products containing nanomaterials that are not measurable with other analytical tools, such as nanoemulsions including unknown refractive index or complex API

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