MULTI-PARAMETER CHARACTERIZATION OF LIPOSOMES BY NANOPARTICLE TRACKING ANALYSIS, DYNAMIC LIGHT SCATTERING, AND ELECTROPHORETIC LIGHT SCATTERING

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Introduction

Liposomes have been used in drug discovery and drug delivery for some time, and the biophysical characterization of these systems and their payloads is critical to understanding and optimizing their fabrication and function. This study looks at optimal conditions for extruding liposomes as well as their stability under different conditions. We` highlight the limit of detection for fluorescently labeled liposomes. Our aim is to further educate the public about the intricacies of liposome formation and characterization as measured by Nanoparticle Tracking Analysis (NTA) from the NanoSight product range and Dynamic and Electrophoretic Light Scattering from the Zetasizer product range within Malvern Instruments.

Nanoparticle Tracking Analysis

NTA utilizes the properties of both light scattering and Brownian motion in order to obtain the particle size distribution of samples in liquid suspension. A laser beam is passed through the sample chamber, and the particles in suspension in the path of this beam scatter light in such a manner that they can easily be visualized via a high sensitivity camera and a 20x objective lens. The camera, which operates at approximately 25 frames per second (fps), captures a video file of the particles moving under Brownian motion within the field of view of approximately 100 µm x 80 µm x 10 µm (Figure 1).

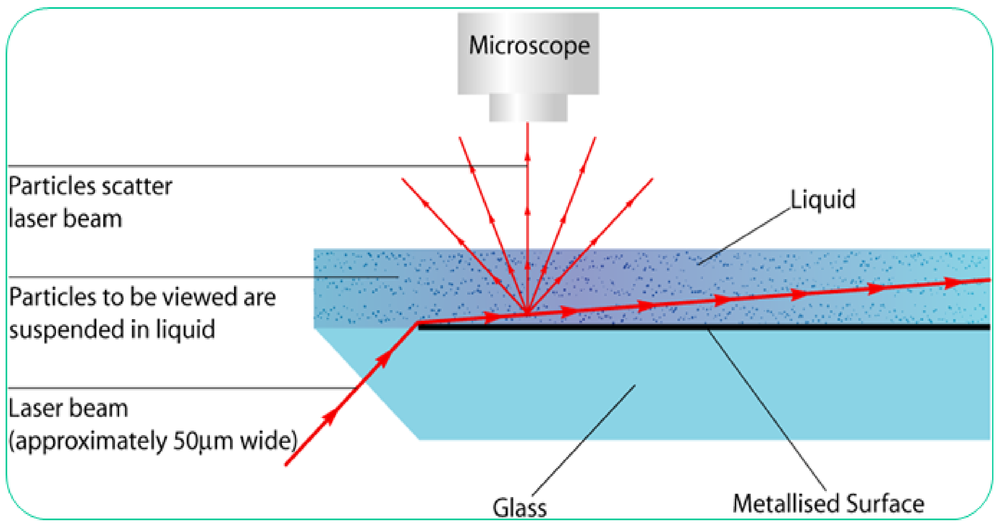


Figure 1. Schematic of the optical configuration used in NTA

The movement of the particles is captured on a frame-by-frame basis. The proprietary NTA software simultaneously identifies and tracks the center of each of the observed particles, and determines the average distance moved by each particle in the x and y planes.

$$D_t = \frac{4K_BT}{3\pi\eta d}$$

Equation 1. Stokes-Einstein equation

This value allows the particle diffusion coefficient (D_t) to be determined from which, if the sample temperature T and solvent viscosity η are known, the sphere equivalent hydrodynamic diameter, d, of the particles can be identified using the Stokes-Einstein equation (Equation 1) where K_B is Boltzmann's constant.

Dynamic and Electrophoretic Light Scattering

The principle of dynamic light scattering is that fine particles and molecules diffuse at different speeds based on Brownian motion. To measure the diffusion speeds, the speckle pattern produced by illuminating the particles with a laser is observed. The scattering intensity at a specific angle will fluctuate with time, and this is detected using a sensitive avalanche photodiode detector (APD). The intensity changes are analyzed with a digital autocorrelator which generates a correlation function. This curve can be analyzed to give the size and size distribution (Figure 2).

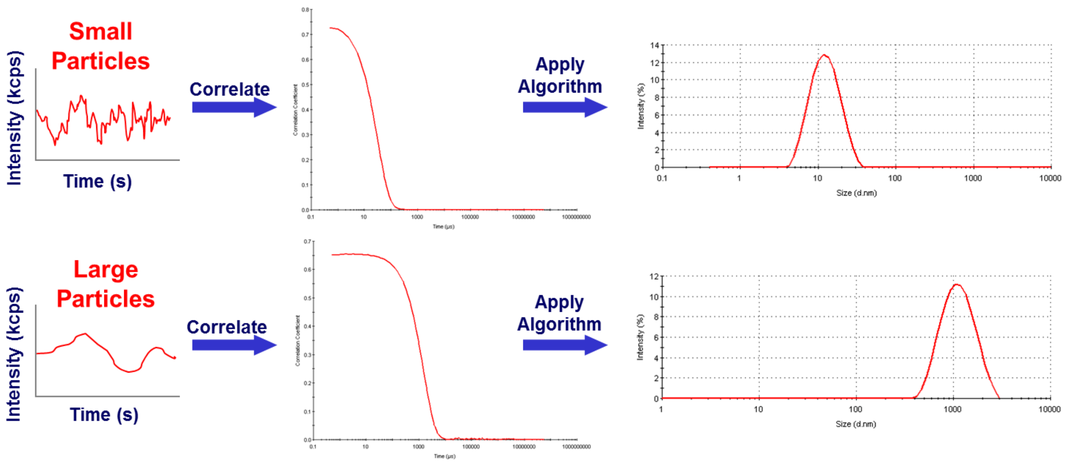


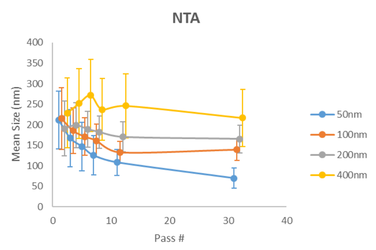
Figure 2. Correlation of Intensity Fluctuation to Size Distribution by DLS

The charge acquired by a particle or molecule in a given medium is its zeta potential and arises from the surface charge and the concentration and types of ions in the solution. The charge or zeta potential of particles and molecules is determined by measuring their velocity while they are moving due to electrophoresis. Particles and molecules that have a zeta potential will migrate towards an electrode if a field is applied The speed (V_P) is proportional to the field strength (E_f) and their zeta potential or electrophoretic mobility (U_E) (Equation 2).

$$U_E = \frac{V_P}{E_f}$$

Equation 2. Electrophoretic Mobility equation

Results



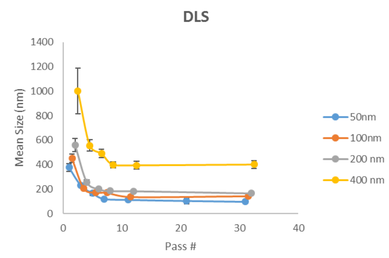
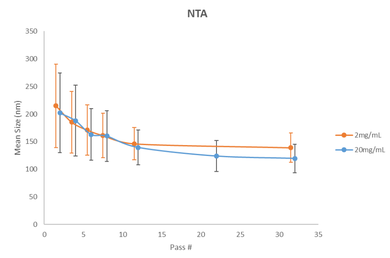


Figure 3. DOPC, 2mg/mL, checking size by extrusion pass number with various pore-sizes



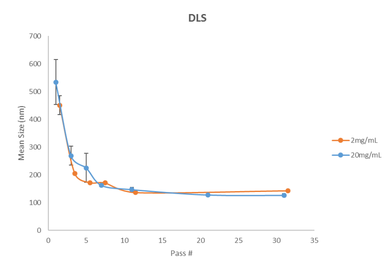
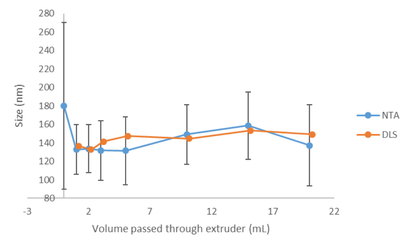


Figure 4. Effect of varying lipid concentration on extrusion sizing by pass # through 100nm pore size



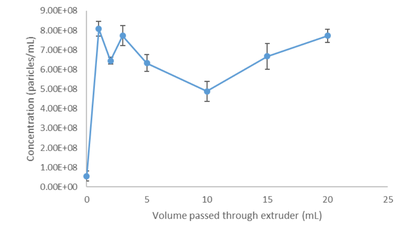
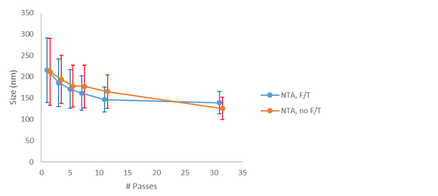
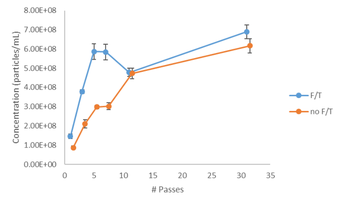


Figure 5. Testing limit of track-etched membrane through 100 nm pore size (each mL passed 11x)





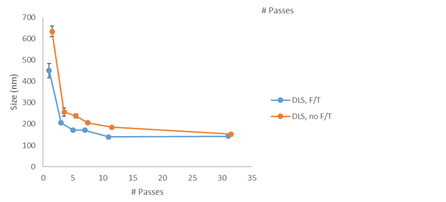


Figure 6. Effect of Freeze / Thaw cycles (5x) on particle size and concentration as extruded through 100 nm pores

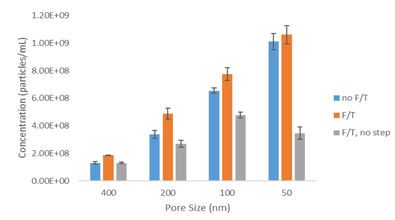


Figure 7. Effect of both freeze/thaw cycles and step-down extrusion on concentration (11x passes/pore size) - NTA

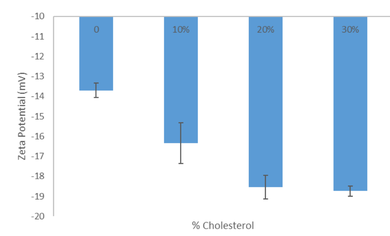


Figure 8. Effect of % Cholesterol on zeta potential (1mM NaCl + 0.1mM MOPS) with diffusion barrier method00

% Rh-DPPE weight	% Labeling efficiency
0.1	70
0.25	113
0.5	121
1	115
2	108
5	95
10	63

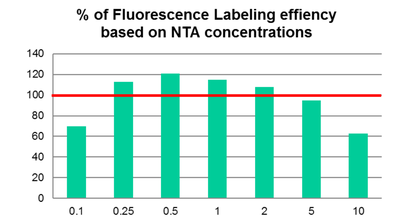


Figure 9. 2mg/mL DOPC with varying mol% Rh-DPPE

Discussion

NTA, DLS, and ELS provided a comprehensive characterization of liposomes across various conditions. Both NanoSight and Zetasizer provide similar techniques because they both rely on the Brownian motion of and light scattering from the particle. Both use the Stokes-Einstein equation and relate diffusion to size (hydrodynamic diameter). In practice, they are quite different, since NTA produces a number-based size distribution and DLS produces an intensity-based distribution. NTA provides particle-by-particle measurement while DLS provides an ensemble measurement. This is further exemplified in the first set of experiments shown here. NTA and DLS confirmed that approximately 11 passes were needed to reach the target pore size (Fig 3).

During the initial passes, DLS reflected the larger particles that were present because of their higher intensity. Once the larger particles were removed, the passes quickly reflected the pore size of the extruder. NTA displayed an initially larger standard deviation in the data because the particle sizes varied more by number.

Neither original lipid concentration nor freeze-thaw cycles had a discernable effect on extruded sizes through different pore sizes (Fig 4 and 6). However, DOPC liposomes subjected to freeze-thaw cycles did have noticeably higher initial concentration when increasing the number of extrusion passes (Fig 6). Robustness of the Nanosizer Extruder by T&T Scientific was demonstrated by the stable size and concentration over 20 mL (11 passes/mL) of extruded DOPC liposomes (Figure 5). There was a noticeable difference in resulting concentration of liposomes subjected to step-down extrusion vs extrusion directly at different pore sizes (Fig 7). This provided milder conditions and higher retention of liposomes. Increasing the percentage of cholesterol in DOPC further displaced head groups in the lipid bilayer causing an increase in the zeta potential (Fig 8). Fluorescence measurements on Rh-DPPE incorporated into DOPC showed that lower percentages could be further measured to determine the true limit of detection (Fig 8). Decreasing % labeling efficiency with higher mol% Rh-DPPE indicates potential fluorophore quenching. Loaded and larger particles potentially offer better centering with inserted fluorescence filter during NTA measurements.

Conclusions

A broad range of characterization information and combination of both NanoSight and Zetasizer systems helped further optimize fabrication and understand the function of liposomes. NTA through NanoSight provided number-based high resolution sizing, accurate distribution profiles, concentration (particles/mL), and fluorescence measurements. DLS provided excellent reproducibility, mean size and PDI measurements over a broad range and non-invasive trend analysis. ELS provided zeta potential as a functionality and stability metric of particles.

Acknowledgments

We would like to thank our collaborators Dr's. Graham Taylor and Nima Tamaddoni at T&T Scientific (Knoxville, TN) for working with us to produce this data. They provided the liposomes and their Nanosizer extruders. NTA, DLS, and ELS analysis was completed at their facility.

✶ Please visit the Malvern booth in the exhibition area for more information and discussion ✶

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