Hook up same-time sizing and encapsulation efficiency of pDNA-LNPs with Stunner AF

Introduction

Lipid nanoparticle (LNP) characterizations can quickly get out of hand. Typically, you'll assess six or more critical quality attributes (CQAs) across multiple instruments with tedious, complex workflows and LNP disruption or dilution. When you add on sizing information gathered from samples one-by-one, development can slow to a crawl.

Following the success of the COVID-19 mRNA-LNP vaccines, there's been more interest in diversifying to other nucleic acid payloads. That includes plasmid DNA (pDNA), which is less expensive than mRNA but easier to transport and can use similar LNP formulations.[1] Platform-based LNP characterization which provides multiple measurements and handles different nucleic acid payloads would simplify development and accelerate adoption of pDNA-LNPs.

Quantifying encapsulation efficiency (EE%) of pDNA-LNPs can be particularly challenging. Disrupting pDNA-LNPs with surfactants can change the payload's isoform which also changes its affinity to the fluorescent dyes common for EE% assays. A single standard curve, therefore, may not work for quantifying free pDNA before disruption AND total pDNA after disruption, leading to incorrect EE% values and more delays, or worse.

Stunner AF hooks up high-throughput UV/Vis, rotating angle dynamic light scattering (RADLS), and fluorescence detection to get EE%, total nucleic acid, free nucleic acid, size, polydispersity, and particle concentration – all in a low-volume, high-throughput, plate-based platform (Figure 1). Best of all, you won't need to dilute or disrupt your LNPs with surfactants to get results-and it works with RNA or DNA.

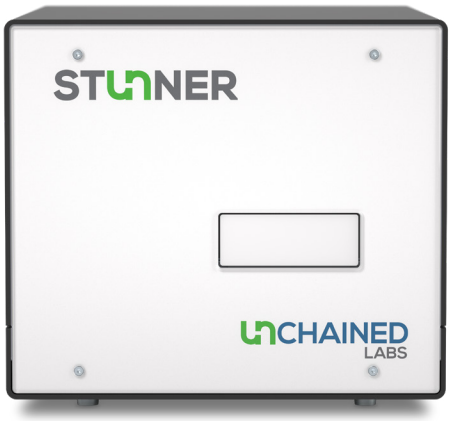

Figure 1: Stunner AF. The ultimate LNP quantification and sizing tool.

Simplified characterization means you can test more samples, faster, to find the optimal pDNA-LNP sooner.

This app note details how Stunner AF simplifies pDNA-LNP characterization, delivering precise results on 5 CQAs in a single read, without surfactants or disruption.

Methods

To make the LNPs, SM-102, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol and DMG PEG-2000 at a 50:10:38.5:1.5 ratio was used at a concentration of 6.2 mg/mL in ethanol. Plasmid DNA (pAAV-Helper, GenScript) was dissolved in 50 mM acetate buffer, pH 4, to a final concentration of 90 µg/mL and a Nitrogen/Phosphorus (N/P) ratio of 6.4. LNPs were synthesized in triplicate on Sunscreen with a Sunny 190X using a flow rate ratio (FRR) of 3:1 (Aqueous:Organic) and total flow rate (TFR) of 10 mL/min. After synthesis, each LNP sample was dialyzed at 4°C overnight into 1X phosphate-buffered saline (PBS).

All metrics were measured in triplicate, either on Stunner AF's Nanoparticle EE or DNA-LNP applications with water or auto-blanking. The mean pre-dialysis total pDNA concentration was compared to the expected output concentration of 67.5 µg/mL for each LNP sample to calculate % yield according to the following equation:

$$\%\ yield = \frac{[total\ pDNA]}{[expected\ output\ pDNA]} \times 100$$

The hydrodynamic size, polydispersity, and the total pDNA, free pDNA, and particle concentrations of the LNP samples were determined post-dialysis. Encapsulated pDNA and EE% were calculated by the Stunner Analysis software from the medians of the total and free pDNA. To prepare the LNPs for free pDNA measurement by fluorescence, Unchained Master Mix was combined with RiboGreen® at a 1:60 dilution to create Working Solution. 5 µL of each LNP sample or reference standard was added to 20 µL of Working Solution. Total pDNA was determined from undiluted LNP samples using deconvoluted A260 values and a concentration factor of 50 (ng/µL) cm.

Stunner AF automatically adjusted for hyperchromicity to account for the impact of encapsulation on pDNA when quantified by UV/Vis. All particle size, PDI, and particle concentration data was collected by RADLS. A buffer viscosity and refractive index of 1.002 cP and 1.334, respectively, at 20°C and the default acquisitions of 7 angles, 5 acquisitions, and 1 second each were used. The Z-average diameter shown was taken at the maximum angle of 162° and the particle concentration was taken from the peak-of-interest (PkOI) of the numbers distribution.

Results

Stunner AF helps you speed through pDNA-LNP characterization by giving you 5 CQAs in a single instrument and one application, reducing hands-on time and simplifying workflows.

The hydrodynamic size and polydispersity (Figure 2A) of triplicate pDNA-LNP samples was 115 ± 2.7 nm with PDIs of 0.15 ± 0.02. The relative standard deviation (RSD) for the size is 2.3% and the PDIs are below 0.2, indicating the pDNA-LNPs did not contain large aggregates.

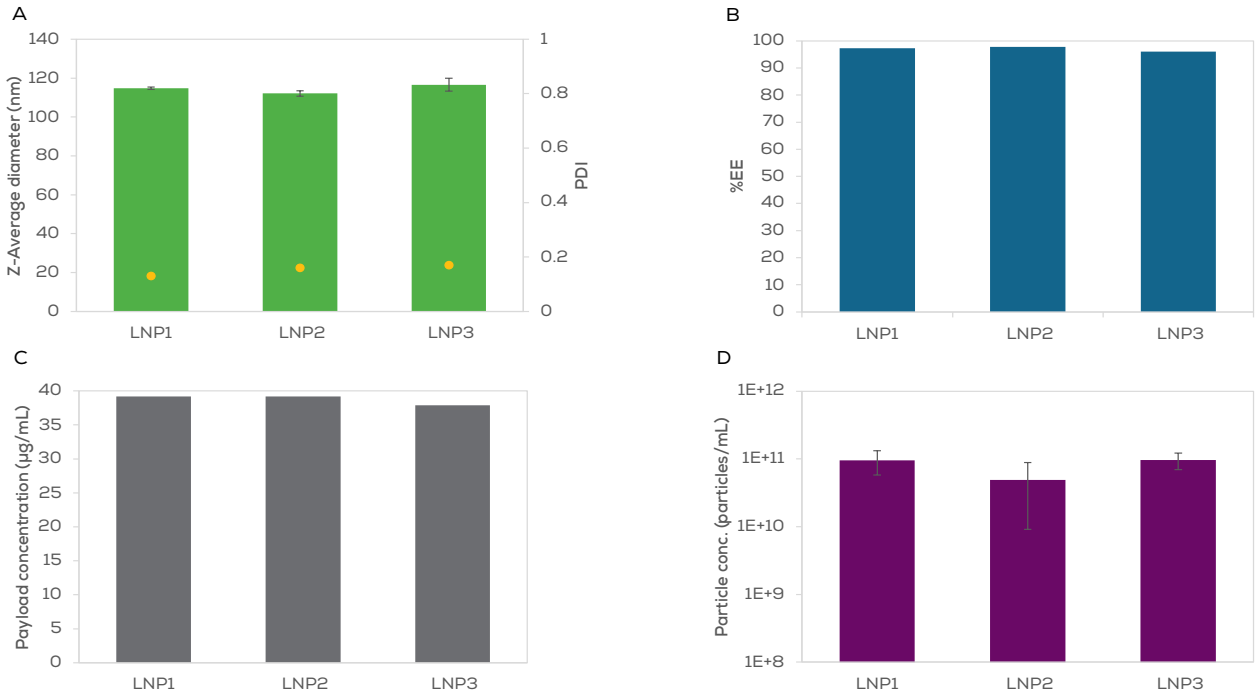

Figure 2: Size, polydispersity (**A**), %EE (**B**), encapsulated payload concentration (**C**), and particle concentration (**D**) of triplicate pDNA-LNP samples made with Sunscreen. Error bars are the standard deviation of 3 replicates measurements on Stunner AF.

Plasmid DNA of different isoforms interact differently with fluorescent dyes, leading to differences in fluorescent output.[2] Disrupting pDNA-LNPs with surfactants, as is required for standard EE% assays, may change the isoform of the payload. This makes determination of %EE of pDNA-LNPs challenging since a single standard curve may not be accurate for pre- and post-lysis nucleic acid quantification. Stunner AF doesn't need a lysis step for measuring the concentration of total pDNA because it uses UV/Vis quantification, which works when the pDNA is still encapsulated.

This eliminates the problem and simplifies the workflow significantly. The %EE of the triplicate pDNA-LNPs made on Sunscreen was impressive, exceeding 96% for all triplicates and averaging 97 ± 0.9% (**Figure 2B**). The encapsulated pDNA concentration was similarly consistent with an average of 38.8 ± 0.8 µg/mL across triplicate LNP fabrications (**Figure 2C**).

Single-angle DLS gets you sizes and polydispersity, but RADLS can also determine the particle concentration, which is linked to dosage, bioavailability, and potency. Stunner AF's LNP apps give you the choice of doing single-angle DLS for fast sizing results fast or RADLS when you want more complete characterization. The average particle concentration of the triplicate LNP fabrications on Sunscreen was 8.0e10 ± 2.7e10 particles/mL (**Figure 2D**).

Sunscreen conserves valuable nucleic acid payloads by providing consistent manufacturing of pDNA-LNPs with reproducible high yields. The % yield for all pDNA-LNPs was 80% or higher with an average of 87± 5.6% (**Figure 3**).

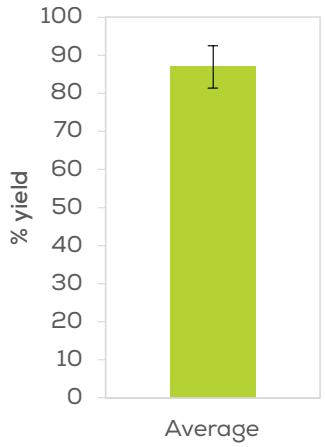

Figure 3: Average yield of triplicate pDNA-LNP samples made with Sunscreen. Error bars are the standard deviation.

Conclusion

Characterizing pDNA-LNPs can be a headache when you're forced to use multiple instruments with complex workflows, especially if you need to create multiple fluorescent standard curves to measure pre- and post-lysis pDNA concentration. Stunner AF lets you determine particle size, polydispersity, payload concentration, EE% and particle concentration from a single application, on one device, in one workflow, without lysis. Sunscreen is Stunner AF's perfect partner, churning out consistent pDNA-LNPs at high-throughput. Together, they can take down any LNP project with less time and effort – on any payload.

References

1. The Expression Kinetics and Immunogenicity of Lipid Nanoparticles Delivering Plasmid DNA and mRNA in Mice. W Zhang, et al. Vaccines. 2023; 11(10):1580.

2. Quantitation of supercoiled circular content in plasmid DNA solutions using a fluorescence-based method. MS Levy, et al. Nucleic Acids Research. 2000; 28(12):e57.

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