Therapeutic nanoparticle prep that meets you where you're at with Sunshine

Introduction

Getting therapeutic nanoparticle drugs to market involves a series of intensive development stages, in which the candidate therapeutic payload frequently needs encapsulating into nanoparticles for performance and efficacy testing. At some of these stages, the nanoparticle formulation itself is the key focus, with researchers looking to tweak and tune the composition of the particle, and the process parameters with which they are produced. The ultimate end goal of this is an optimized nanoparticle delivery vehicle for a precious therapeutic payload (typically a nucleic acid construct such as DNA or RNA), and a dialed-in process for manufacturing them which can be transferred into GMP for clinical trials (Figure 1).

But before optimizing the nanoparticle delivery package, the development of the payload requires frequent encapsulation of candidate nucleic acid constructs in order to test in vitro and in vivo performance to identify successful candidates. This is typically done with a standard nanoparticle formulation that needs to give quick, consistent encapsulation and delivery into cells. Synthesizing individual, unique RNA or DNA payload candidates is an expensive and time-consuming process, and therefore every molecule matters – so when it comes to getting the prospective payload encapsulated, low volume and high efficiency remain key.

Sunshine offers an industry leading combination of automation, re-usable microfluidics and production volume flexibility for the synthesis of therapeutic nanoparticles such as lipid nanoparticles (LNPs), polymer nanoparticles (PNPs) and liposomes. Offering both continuous flow nanoparticle production and automated generation of sequential small-volume nanoparticle samples, Sunshine suits development needs from early stage research pinning down the right payload through to pre-clinical process optimization of the final drug product.

There are 3 different Sunshine setups available to provide even more flexibility when it comes to picking the system that's got the goods for the task at hand (Figure 2). As needs change over time, Sunshine can be converted from one configuration to another, allowing for the addition of features without the need to buy an entirely new instrument.

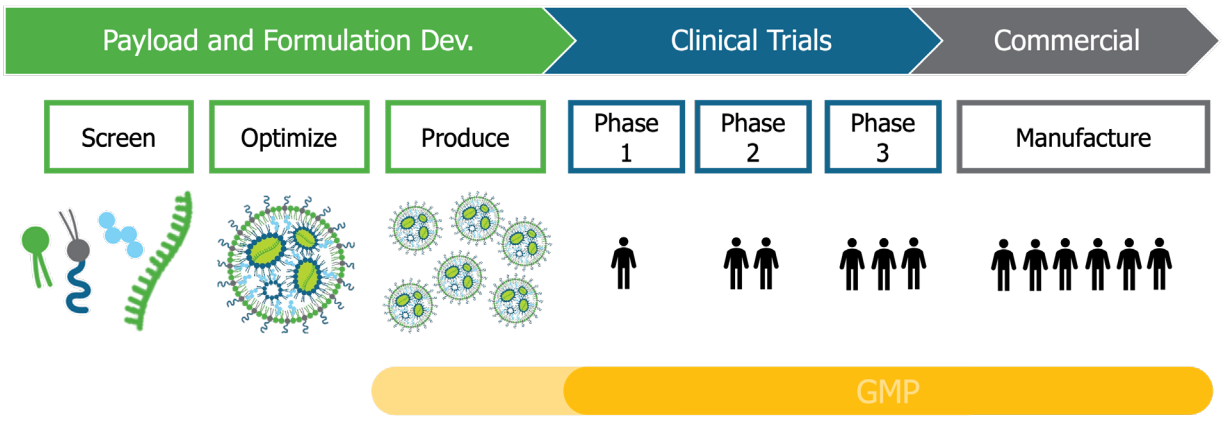
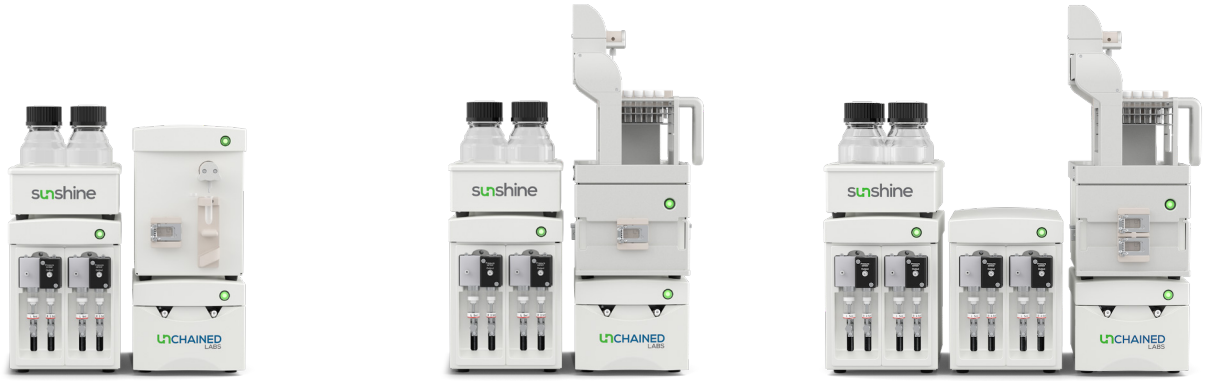


Figure 1: Nanomedicine drug development process.



Sunshine Single | Sunshine Lite | Sunshine

Figure 2: Hardware configurations for Sunshine Single, Sunshine Lite, and Sunshine.

With speed and simplicity in mind, Sunshine Single's dedicated software guides the user through the setup and execution of each experiment, allowing anyone in the lab to walk up and run a single nanoparticle formulation to get payloads encapsulated pronto. The shortened flowpath of Sunshine Single allows for the use of experiment volumes down to 400 µL, while maintaining high sample yields – perfect for the early stages of payload development where rapid performance testing of your construct is critical, but every drop of nucleic acid is precious. Sunshine Single offers a new entry point into the Sunny Suite (which includes Sunscreen for high-throughput nanoparticle formulation screening, and Sunbather for GMP production).

Sunshine Lite brings automated collection to the table, along with continuous production mode. Queue up experiments to explore flow conditions for your chosen formulation, dial in the Sunny mixing chip, and when you're ready you can produce bulk volumes of particles by running the pumps in continuous mode. This uses the same flow rates and flow ratios used for your small-volume automated experiments, matching particle quality from 1 mL samples up to liters of product.

In this application note, LNPs produced on each of Sunshine, Sunshine Lite and Sunshine Single are presented to demonstrate side-by-side system performance.

Results

Size

PolyA-SM-102 LNPs were synthesized on each of Sunshine, Sunshine Lite, and Sunshine Single across a range of flow rates. The data from all 3 instrument configurations show particles ranging in size from around 170 nm at the lowest tested flow rate of 2 mL/min, down to 85 nm at a flow rate of 12 mL/min. For the Sunny 190 X (which was used for all experiments presented), particle size decreases as flow rates increase up to a flow rate of around 6 mL/min, at which point particle size remains consistent with further increases in flow rate. This is due to the transition of fluid mixing from purely laminar flow at <6 mL/min to more chaotic at >6 mL/min. At all flow rates tested, particle size is consistent within standard error between the Sunshine, Sunshine Lite and Sunshine Single instrument setups.

Throughput

The re-usable Sunny mixers are cleaned in place by all Sunshine systems after every experiment. This means going straight onto the next experiment instead of wasting time manually cleaning the system, allowing quick-fire generation of samples for characterization and performance testing. By sequentially automating the 6 different flow rates on Sunshine and Sunshine Lite, each set of 6 samples was ready for collection after 10 minutes, all from a

single reagent loading step. Each individual experiment on Sunshine Single took less than 5 minutes to complete, and the total of 54 experiments shown in (Figure 3) were carried out on Sunshine and characterized on Stunner in a single day, using a single Sunny 190 X.

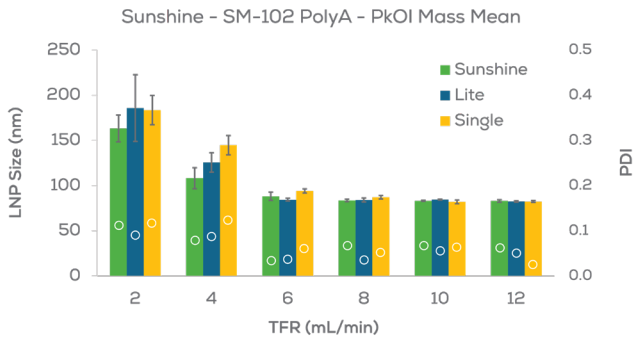


Figure 3: Flow rate screen comparison of each Sunshine instrument setup.

Encapsulation Efficiency

It's critical for any nanoparticle production process that the particles formed effectively encapsulate the target payload, which in the case of LNPs is typically a nucleic acid. For these experiments, Polyadenylic acid (PolyA) was used as a substitute for coded RNA. Due to the consistency of mixing created by both the automation of experiments and the precision engineering of the Sunnies, the LNPs synthesized on each Sunshine setup consistently showed extremely high encapsulation efficiencies of >98% (Figure 4). To get encapsulation efficiency, size, PDI, particle and payload quant from a single instrument, check out Stunner.

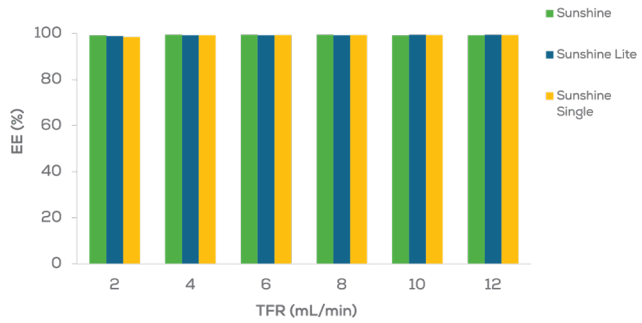


Figure 4: Average EE% for each of the samples shown in the flow rate screen comparison.

Sample volume

As well as high encapsulation efficiency to ensure that every precious molecule of nucleic acid cargo is utilized, it's also desirable to only use what's needed for downstream testing. That's why all Sunshine setups can run experiments down to 400 µL (100 µL of lipid mix in ethanol + 300 µL of RNA in buffer). (Figure 5) shows a particle size and PDI comparison between Sunshine and Sunshine Single (Sunshine Lite shares the same flowpath lengths as Sunshine, and is omitted here as identical). The data shows decreasing collected sample volume from 1000 µL to 400 µL, with (+) or without (-) 100 µL head and tail cuts. This is visualized in (Figure 6).

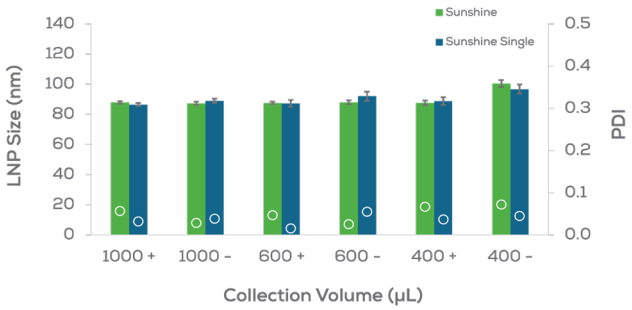


Figure 5: LNP samples collected with (+) and without (-) 100 µL head and tail cuts with decreasing sample volume.

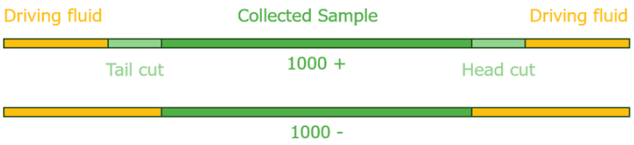


Figure 6: Visualization of a 1000 µL nanoparticle sample in tubing with and without 100 µL head and tail cuts. For 1000+, the total sample volume was 1200 µL, of which 1000 µL was collected.

For Sunshine, experiments were run individually for a direct comparison to Sunshine Single. When automating multiple experiments on Sunshine or Sunshine lite use sample volumes ≥1 mL to minimize the impact of sample dispersion with the driving fluids used.

At larger collection volumes, particle quality is excellent for all experiments. At the lowest sample volume of 400 µL, a small increase in particle size can be observed, likely due to the impact of dispersion of the sample with driving fluid. As the data shows, this can be easily mitigated by including a head and tail cut.

Conclusion

Sunshine instruments offer solutions for researchers at every stage of development with the ability to flex the system feature set to suit. Each configuration of Sunshine produces the same high-quality nanoparticles across all flow rates and volumes tested, and share features such as automated flowpath cleaning, low volume particle production and a range of Sunny mixer types to suit any application - all of which are 100% re-usable, saving on both running costs and plastic waste. Those Sunnies, along with the pumping technology, are common to all Sunny Suite systems, allowing easy transfer of process parameters to ensure consistent particles every time.

Methods

To make the LNPs, the Spikevax formulation (SM-102, 1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC], cholesterol and DMG PEG-2000 at a 50:10:38.5:1.5) was used at a concentration of 6.2 mg/mL. PolyA (Sigma Aldrich) dissolved in 50 mM acetate buffer, pH 4.50, to give a Nitrogen/Phosphorus (N/P) ratio of 6. LNPs were synthesized in triplicate across 3 separate experiment runs. Error bars shown are the standard deviation of the particle size of the three samples, where each size measurement is an average of 3 separate DLS measurements.

For all experiments, a flow rate ratio (FRR) of 3:1 (Aqueous:Organic) was used. 50 mM acetate buffer, pH 4.0 was used as a driver fluid for the aqueous phase and ethanol was used as a driver fluid for the organic phase, and a Sunny 190 X was used to produce the LNPs.

For the data shown in Figure 3, the total sample size for each LNP experiment was 1000 µL with a 100 µL head and tail cut, giving a total collected volume for each sample of 800 µL. For Sunshine and Sunshine Lite, experiments across all flow rates were automated, with lipid formulation and PolyA being loaded into 5 mL sample loops via a single loading step, with experiments run from fastest to slowest. Sample loops were overloaded by 10%. For Sunshine Single each experiment was run individually.

For the data shown in Figure 6, all experiments were run individually for a direct comparison of performance between Sunshine and Sunshine Single. A flow rate of 10 mL/min was used for all experiments.

All particle size and PDI data was collected by rotating angle DLS (RADLS) measurements using a Stunner, measuring at 7 different angles. The size data displayed is the mass mean PkOI. Samples were measured in triplicate. Total RNA quantification was carried out by UV/Vis also on a Stunner.

EE% was carried out by Invitrogen Quant-it Ribogreen assay following manufacturer's instructions. Samples were measured intact and after lysing with 1% Triton X, and fluorescence was measured by FLUOstar Omega plate reader.

Unchained Labs
4747 Willow Road
Pleasanton, CA 94588
Phone: 1.925.587.9800
Toll-free: 1.800.815.6384
Email: info@unchainedlabs.com

Rev A