Wolfe Laboratories — A Subsidiary Of — Pace Analytical® Life Sciences

Oligonucleotide Lipid Nanoparticle Production and Characterization

Introduction

Clinical success of systemically administered oligonucleotides and gene therapies (e.g., siRNA, mRNA, CRISPR/Cas9) relies on appropriate formulation to ensure safety, transport to site of action, and efficacy. Lipid nanoparticles comprise a promising non-viral formulation strategy for oligonucleotides, exemplified by recent FDA approval of Patisiran™ (Alnylam). Traditional batch syntheses of liposomal materials typically provide poor control over size and dispersity, necessitating laborious post-process size reduction techniques (sonication, extrusion, etc.). In addition to complicating early-phase discovery and contributing to substantial materials loss, these size reduction and homogenization steps can be detrimental to oligo integrity and can also be problematic for later-phase scaleup and manufacturing. Microfluidic mixing of oligonucleotide and lipid components (Figure 1) overcomes these obstacles by exercising control over the characteristic mixing timescale (τ_m) of LNP nanoprecipitation.

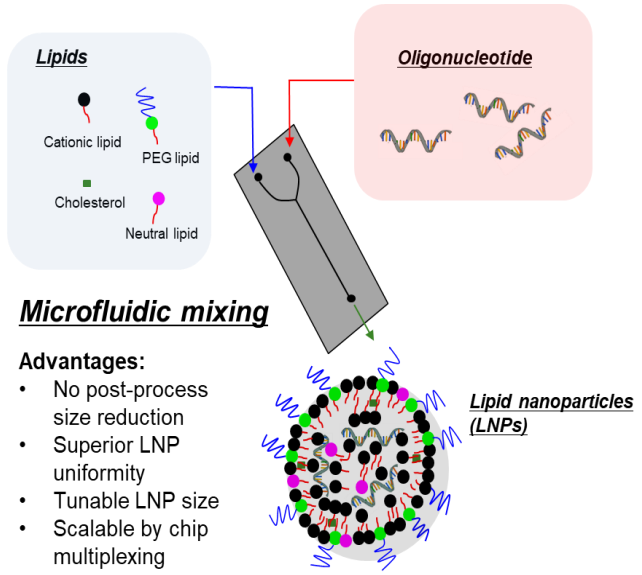

Figure 1: Overview of a microfluidic mixing process to synthesize oligonucleotide-containing LNPs (in contrast to liposomes, LNPs are not observed to contain a contiguous lipid bilayer).

LNP Production

1. Microfluidic vs. conventional mixing Wolfe Laboratories has developed a continuous microfluidic process to synthesize oligonucleotide-containing LNPs. To demonstrate this process, we encapsulate a single stranded 24-mer oligonucleotide using a four-part mixture of cationic lipid, PEG-lipid, neutral lipid, and cholesterol. Our process utilizes the rapid mixing rate (low τ_m) of aqueous oligonucleotide and ethanolic lipid streams afforded by microfluidic mixing. Under such conditions, complete mixing occurs on a faster timescale than particle aggregation ($\tau_m < \tau_{agg}$), leading to sub-100 nm, monodisperse LNPs without the need for post-processing (Figure 2). On the other hand, LNPs synthesized by mixing the same aqueous/ethanol streams (at the same flowrates) with a conventional T-junction possess larger size, polydispersity, and run-to-run size variation due to a slower and less controlled mixing rate often time leading to particles lacking needed drug substance homogeneity and stability.

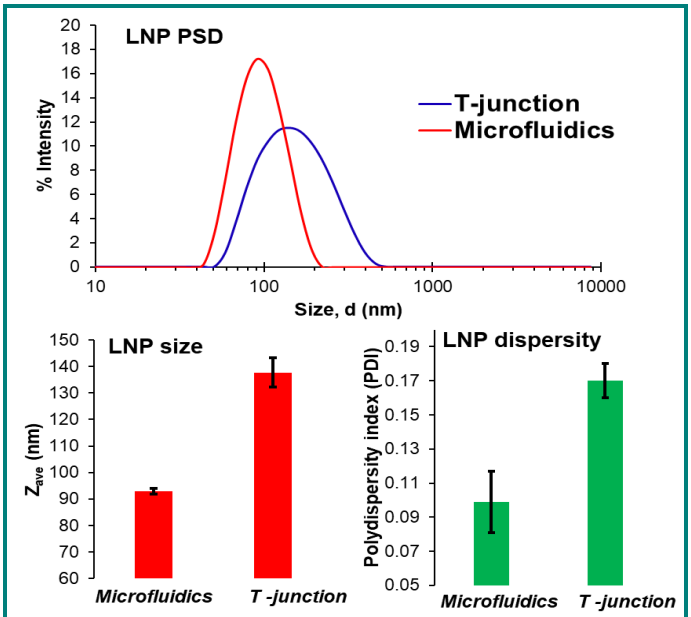

Figure 2. Top: Dynamic light scattering (DLS) particle size distributions of oligo-LNPs synthesized using microfluidic mixing or conventional T-junction mixing (equal flowrates for both experiments). Bottom: Size and polydispersity indices of microfluidic/conventionally mixed LNPs; each bar plot represents the average of 3-4 independent runs (mean ± SEM).

2. Size-controlled LNPs using microfluidic flowrate LNPs produced with microfluidic mixing exhibit smaller sizes as the total flowrate is increased (Figure 3, top). This observation is consistent with our understanding of how microfluidic mixing rates increase at higher flow rates due to enhanced chaotic advection. We can utilize this dependence to reproducibly tune the size of LNPs. For instance, when the total flowrate is alternated between

'low' and 'high' flowrates, the size of the LNPs alternates between ~140 nm and ~90 nm, respectively (Figure 3 bottom) with statistically insignificant differences in polydispersity.

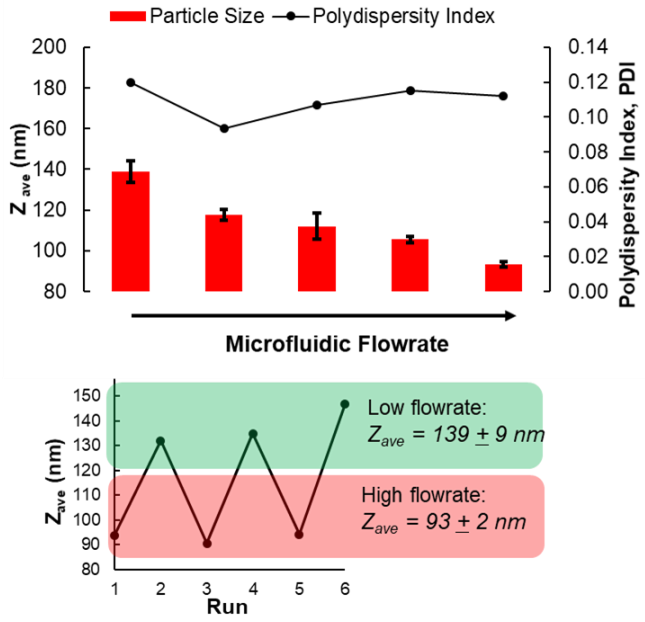

Figure 3. Top: Dependence of LNP size and PDI on total microfluidic flowrate. Bottom: Alternating the flowrate between high and low flowrates reproducibly leads to small and large LNPs, respectively.

LNP Characterization

1. Encapsulation Efficiency of LNPs We measure unencapsulated oligonucleotide content, $O_{unencap}$, of LNPs in situ by fluorescent enhancement of an intercalating dye (Figure 4). Total oligo content, O_{total}, is similarly measured after chemical disruption of LNP. An LNP encapsulation efficiency (EE) of 90% is routinely achieved with our

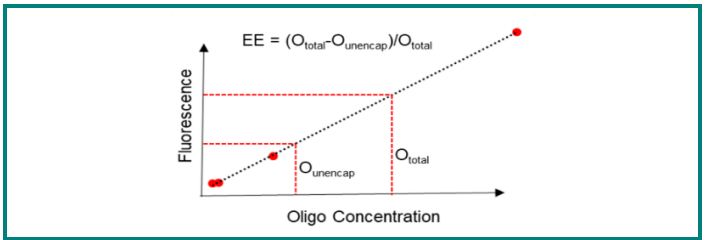

Figure 4. Encapsulation efficiency of LNPs via fluorescence measurements of intercalative dyes

microfluidic process for various N/P ratios (ratio of lipid headgroups to oligonucleotide phosphate groups).

2. Content and Purity Stability indicating analytical methods for lipid and oligo content and purity are critical to ensuring appropriate formulation/process conditions and drug substance storage stability. Our approach is to develop stage appropriate, UPLC-based, custom analytical methods for lipid and oligo components. For lipid analysis (typically weak chromophores), we utilize detection by evaporative light scattering detection (UPLC-ELSD) with reversed phase separation that is tailored to the specific LNP composition. For oligonucleotides, ion-pairing reversed phase (IP-RP-LC) offers the chemical selectivity and resolution to separate and quantitate synthesis, process and storage-related impurities. IP-RP development relies on assessing a wide range of RP columns and mobile phase components to accommodate different oligo chemical modalities (stabilizing modifications and size). Figure 5 displays representative chromatograms for each of these techniques.

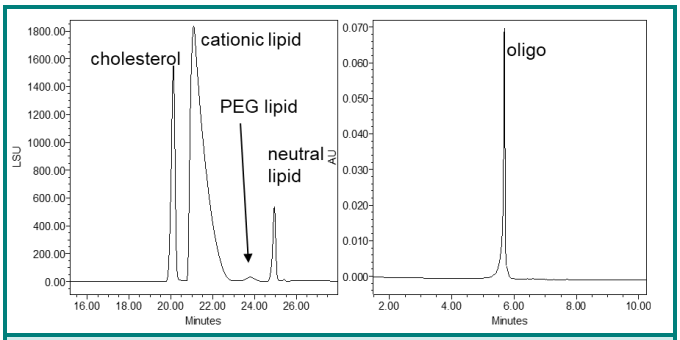

Figure 5. UPLC content analysis of LNPs: lipids (left, UPLC-ELSD) and oligonucleotides (right, IP-RP-UPLC).

3. Size and Integrity of LNPs Process control and therapeutic efficacy are dependent upon a defined and consistent LNP size and predictable stability. During the formulation process, under various accelerated stress conditions and during real time stability programs we evaluate the size and integrity of LNPs through a combination of DLS, Zeta-potential, and SEC-MALS. For example, lipid compositions that lead to unstable particles rapidly coalesce into a larger polydisperse aggregate. Figure 6 demonstrates a short temporal DLS study of a LNP evaluated to be stable vs. an LNP that is unstable and prone to unwanted aggregation under the same storage conditions.

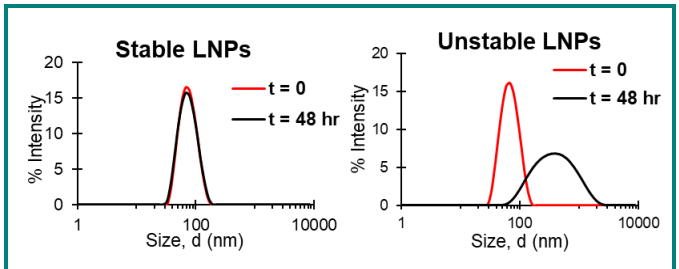

Figure 6. DLS particle size distributions of a stable LNP formulation (right) and an unstable formulation (right), which aggregates over the course of 48 hours.