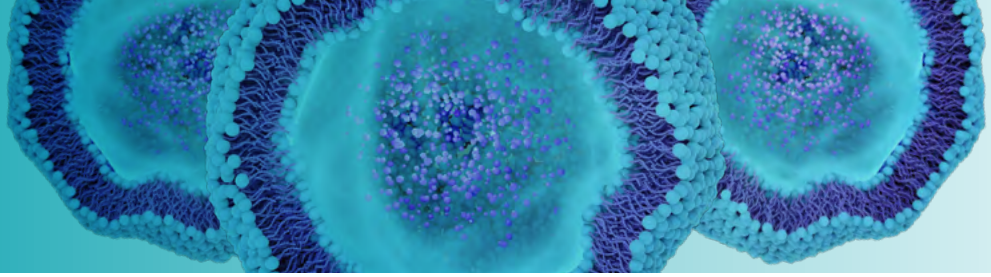
ReciBio Pharm



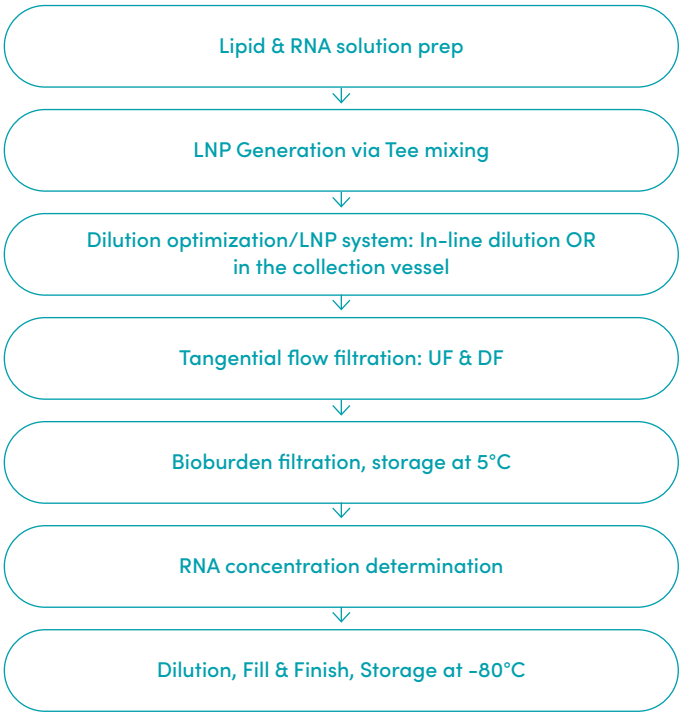
Laminar and Turbulent mixing techniques for Lipid nanoparticle formulations scale up approach

xRNA as a therapeutic or vaccine modality requires a vehicle to protect it as it travels to its site of release and subsequent translation. While nucleic acids offer promising therapeutics for many applications, they present challenges in terms of stability and efficacy. Non-viral delivery systems such as polymeric or lipid nanoparticles (LNPs) act as excellent vehicles for nucleic acids to improve their *in vitro* transfection and *in vivo* stability. There have been several successful commercialized non-viral RNA drugs that have made it to market such as Onpattro® and the two SARS2-COVID vaccines from BioNtech/Pfizer and Moderna. The production of two COVID-19 vaccines in less than a year with fast-track emergency approval by FDA was a milestone that drastically changed outcomes for COVID-19 patients, particularly among the most vulnerable. For this white paper, we will focus on the formulation and process development of lipid nanoparticles.

LIPID NANOPARTICLE FORMULATION

LNP formulations are composed of four components, which is an ionizable lipid, phospholipid, cholesterol, and pegylated lipid. The ionizable lipid plays a crucial role in the encapsulation and the eventual release of the nucleic acid payload. This lipid will become positively charged when mixed in an acidic buffer below its pKa, promoting electrostatic interactions with the negatively charged nucleic acid. Once exchanged into a neutral buffer the ionizable lipid will lose its positive charge but the LNP will remain stable with the help of the other structural lipids. Furthermore, this ionizability promotes the release of the payload when the formulation reaches the acidic environment of the endosome, causing destabilization of the endosome and the LNPs. The other excipients are largely complimentary or structural lipids. The phospholipids and cholesterol help maintain the structural integrity of the LNP. Once mixed, they will form a bilayer that protects the cargo and forms the particle. The phospholipid could be either saturated or unsaturated (DSPC or DOPE). The fourth component is the pegylated lipid which typically makes up the smallest portion of the LNP. Adjusting this lipid will tune the size and poly dispersity index (PDI) of the formulation, while also providing steric hinderance to protect the formulation in circulation. Particle size of the nanoparticles play an important role in the uptake of the LNP into the cell or its clearance by the reticuloendothelial system (RES). The size is also dependent on the route of administration. The smaller and more homogenous the particles are, the better their uptake into the cell, particularly if through a systematic administration. With other routes of administration, larger particles can be better for RNA/DNA delivery. A low PDI value is an indication that the formulation is homogenous and the size reflected on the dynamic light scattering technique (DLS) exists in one population, which is desirable. Moreover, the pegylated lipid is also important for cold storage stability, helping to prevent particle aggregation during the freezing process.

Non-viral drug product formulations, specifically lipid nanoparticles, are easier to manufacture than viral vector based therapeutics and typically have a lower cost of goods to produce, making them an attractive delivery system for advanced therapeutics. The process of particle formation at the precise concentration includes the steps listed below:



HOW THESE LNPs ARE MADE

LNPs are produced via nanoprecipitation process when an aqueous solution containing the payload (e.g.: RNA) at, typically, low pH is mixed with an ethanolic solution containing the lipid components. When mixed with an acidic buffer, the ionizable lipid becomes positively charged and electrostatically interact with the negatively charged nucleic acid. This promotes encapsulation of the nucleic acid within the LNP core. The other lipids then form a layer around the encapsulated nucleic acid to protect it in solution. This can be accomplished through several different methods such as pipette mixing, microfluidics, tee-junction, and coaxial jet-mixing.

Manufacturing LNPs with precise critical quality attributes (CQAs) at small scale is possible once the formulation optimization and selection is final. However, the complexity increases in magnitude as you scale up from formulation development (small scale) to process development scale to manufacturing scale. At small scale, a Cytiva Ignite™, Unchained Labs Sunscreen™ microfluidics, a tee-junction mixer or a DIANT® Discovery jet-mixer can generate batches with similar physical parameters post mix. Downstream processes involve a mixture of dialysis and/or centrifugal concentration. Final testing of the CQAs panels is identified to guide the scale up process characterization.

This all changes as you scale up, particularly in gene editing therapeutics where large dosing volumes are required. The scale up in this application presents more challenges both for the mixing and TFF concentration & diafiltration steps, and the upstream mixing process needs to be precise, scalable with high reproducibility as well.

The complexity of the scale up process stems not only from the dynamic nature of LNP formulation with excipients that have complex purity profiles over long storage and volatile stability profile, but also from the nucleic acid hydrolysis process in acidic buffer at room temperature.

UPSTREAM MIXING PROCESS

There are critical process parameters (CPPs) that affect the critical quality attributes (CQAs) of LNP formulations. These include total flow rate (TFR), fractional flow rate (FFR), dilution factor and dilution method (in-line or off-line).

TFR may be the most important mixing process parameter for LNP manufacturing. This not only will determine how fast you can manufacture the LNPs, but also plays a major role in the size, PDI and encapsulation of the particles. At slow flow rates larger particles and less monodisperse solutions are observed. However, with increased speed, the particles will begin to decrease in size and become more monodisperse. It is important to characterize a product at several flow rates to determine at which flow rate can achieve a small and uniform LNP solution.

FFR is another critical parameter in LNP manufacturing. This is defined as the flow rate ratio between the aqueous and ethanol phase. This parameter is important for the particles' size and encapsulation.

The dilution factor and method also play a pivotal role in the mixing process. This step is downstream of the initial mixing step and is used to tune the intermediate concentration going into the UF/DF steps of the process but can also be used to stabilize the LNPs.

PROCESS DEVELOPMENT STRATEGIES TO MANUFACTURE LNP BATCHES

Laminar and turbulent mixing are the two main types of mixing used regardless of the technology adopted.

LAMINAR FLOW AND MICROFLUIDICS

Microfluidic devices follow laminar flow with Reynolds number (Re< 100). Laminar flow allows for the fluid to move orderly in regular path. This mixing allows production of controlled homogenous nanoparticles. Microfluidic ensures batch to batch reproducibility after formulation parameters are determined.

Spark and Ignite are two microfluidics units that are manufactured and mass produced by Cytiva. These two mixers simplified small scale LNP production. The mixers have a maximum total flow rate of 12 mL/min and can produce very small batches for effective formulation screening. This allows for fine tune the size, PDI of the nanoparticles while maximizing the percent encapsulation (%EE) with very little material. These two systems work well at very small scale, but as you scale up, they impose scalability issues.

Cytiva developed next generation microfluidic mixers (Blaze/Blaze+). The two units can manufacture larger batches compared to the ignite with faster mixing rates to reduce processing time and guarantee quality of the product. These mixers are more suited for process development labs. The two mixers come with the option of two different chip designs that can accommodate maximum flow rates of 60 and115 mL/min. Cytiva also developed a GMP mixing systemthat allows mixing at relatively higher flow rates compared to the Blaze with a maximum total flow rate of 200 mL/min while maintaining laminar flow within the microfluidic chips.

In the three mixers with total flow rates of 115 mL/min and 200 mL/min, respectively, Cytiva model supports a scale out model with a continuous mode of mixing irrelevant of the batch size.

The scale out model works well if the formulation is stable and if the payload is not highly susceptible to degradation while at RT.

The microfluidic technology championed by Cytiva has revolutionized dynamic formulations 'production and consistency, but this technology comes with its own limitations, such as environmental waste that the chips impose. Such limitations include high cost of single use cartridges, cartridge to cartridge variability, single supply chain, standalone mixers at different batch scale and long mixing time for larger batches with payload-limited stability.

TURBULENT MIXING AND SCALING UP

Turbulent mixing is described as irregular flow of solution in a path with continuous changes in velocity and pressure and in an LNP formulation, the two solutions are introduced to each other at high velocity (TFR) over a limited surface area (100<Re<2000). In turbulent mixing, there are 3 regions that represent degree of mixing in a junction as shown in Figure 3.

There are two major turbulent mixers currently, Tee and jet mixers.

Figure 1. Nanoassemblr™ Classic mixer (Cytiva Lifesciences 2024)

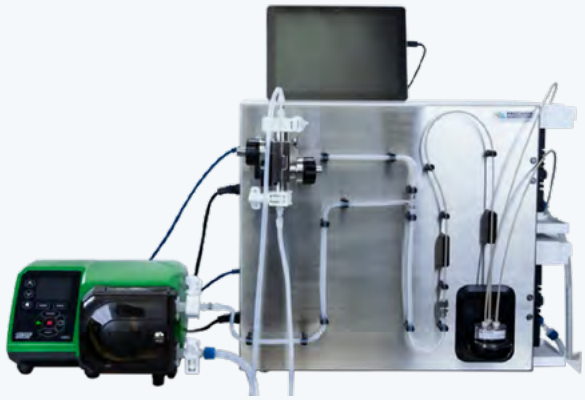


Figure 2. Nanoassemblr™ GMP system (Cytiva LifeSciences, 2024)

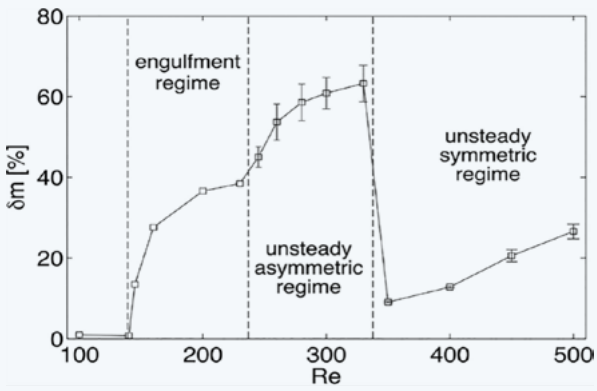


Figure 3. Degree of mixing as a function of Re. Adapted from Ref (Camarri, S., et al, 2020)

TEE MIXERS

During the pandemic, the leading technology that was utilized to produce the COVID-19 vaccine Comirnaty® was based on tee mixing. Tee mixers have a simple geometry with two solutions flowing in the same direction toward the impingement zone. Tee mixing relies on concepts of linear velocity and Reynolds numbers to produce bulk LNP formulations. The main advantage of the tee mixer is the use of speed (high total flow rates) to generate homogeneous nanoparticles in a short time. Advantages of tee mixers are the ability to use different pump systems such as HPLC Knauer pumps or any positive displacement pumps. This does not restrict the user to a certain mixer, reducing the cost with minimum footprint. Turbulent mixing at higher flow rates lead to faster mixing which reduce time when scaling up.

Tee mixers produce bulk formulations, which can consist of more than one size population with larger PDI. More formulation and process development are required before scaling up to meet the CQAs achieved at small scale production. This can be achieved by calculating Re associated with the tested flow rates to pinpoint the sweet spot for the total and fractional flow rate.

JET MIXERS

Jet mixer technique produces LNPs by introducing two fluid streams flowing in the same or opposite direction at high Re numbers increasing the interfacial area. The flow dynamic by which tee mixer and jet mixer is based upon is turbulent mixing. However, each of these bulk mixing techniques have impact on the physical and biological characteristics of formulations that requires another paper to discuss.

A jet mixer is made up of coaxial cylindrical tubes where an aqueous solution and an organic solution are injected through the inner and outer tubes, respectively.

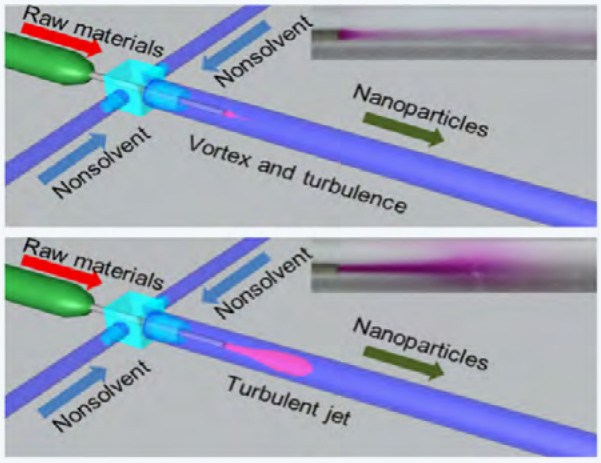


Figure 4. Schematic illustration of coaxial turbulent jet mixer. A) turbulent induced by vortex and, B) turbulence induced by jetting. (Jong-Min, L., et al, 2014)

An example of coaxial turbulent jet is coflow mixer technology produced by DIANT pharma Inc. DIANT jet mixer is composed of two concentric tubes, with the organic solution injected in the same flow direction as the aqueous solution and in the chamber these two form a jet induced by high flow rates.

Production of clinical and commercial batches require large scale production. One approach that has been implemented during COVID-19 vaccine production was parallelization where several Knauer skids were utilized.

Figure 5: Schematic Illustration of DIANT coaxial turbulent jet mixer.

DIANT pharma approach can either be a scale up or scale out approach. Their development is pump based dependent. You can achieve higher flow rates with more powerful pumps (scale up) or through keeping the same flow rates but through longer flow duration (scale out).

DIANT developed a small-scale jet mixer skid: DISCOVERY® that utilizes the same jet mixer used in the scale up equipment; LaRU® skid. LaRU can either be integrated as a unit within continuous manufacturing process or as standalone unit. The DIANT mixer does not utilize disposable parts, but stainless-steel parts which has higher compatibility with solvents. Both Discovery and LaRU mixers produced LNPs with similar CQAs to those produced with microfluidics and with Tee mixers at ReciBioPharm facility in Watertown, MA.

As any other technology, DIANT jet mixers have its own limitations. One of these limitations is large volume requirement per each experiment due to the long flow path before the two streams meet to form the jet. Waste is required at the beginning of the mix to ensure consistent flow. The mixer needs to be cleaned between runs and if the system is used in manufacturing suites and for GMP production, the system would require cleaning verification and validation.

LaRU has a large footprint that can be an issue to process development labs, but if integrated within the continuous manufacturing process with the TFF single pass as part of the skid, this reduces number of unit operations within the design.

At ReciBioPharm, we acquired LaRU® as part of the continuous manufacturing train and Discovery® skid for PD lab. We compared the three mixing technologies mentioned in this paper, using LNPs with fluc mRNA manufactured in house to show translatability between the mixing technologies as shown in the Table 1 and Figure 6.

Table 1:

Mixer type	Tee mixer			Jet mixer			Blaze		
Process Parameters	Size (nm)	PDI	%EE	Size (nm)	PDI	%EE	Size (nm)	PDI	%EE
Post mixing	60.47	0.05	94	61.39	0.07	91	60.6	0.04	N/A
Post Exchange buffer	61.55	0.02	N/A	58.71	0.03	N/A	58.7	0.04	N/A
Post Filtration	62	0.02	N/A	58.45	0.03	N/A	58.5	0.03	N/A
Pre-freeze	63.85	0.02	95	59.91	0.05	91	59.9	0.05	95
Post freeze	71.31	0.06	N/A	68.38	0.05	N/A	60.6	0.03	N/A

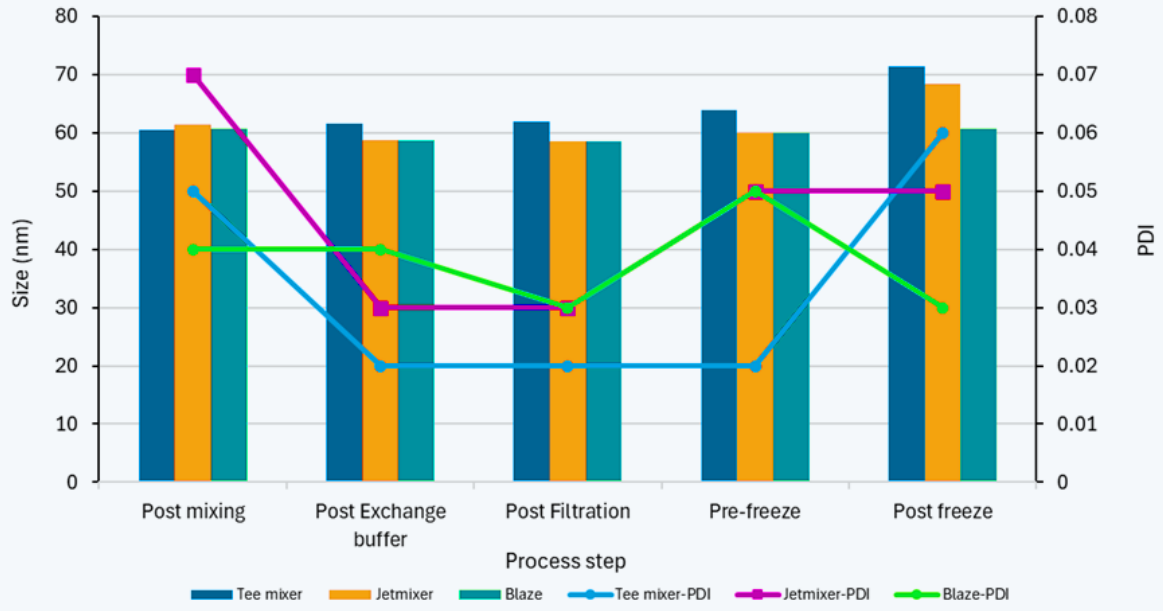


Figure 6: Particle size and PDI results generated from tee mixer, jet mixer and microfluidic Blaze using fluc mRNA as the payload

CONCLUSION

As shown in this white paper, both laminar flow presented by microfluidic technology and turbulent flow presented by jet and tee mixers can be employed in LNP manufacturing at small scale, process development and manufacturing scale, while maintaining drug product CQAs. While laminar mixing provided microfluidic technology as well as other iterations of microfluidics, maintain narrow distribution without changing the consistency of the particles, they are still limited by being a scale-out approach that can significantly increase production time and costs. In comparison, turbulent mixing can allow for a scale-up approach through linear velocity and Reynolds number by experimenting with flow rate and tubing ID. Additionally, different types of pumps and mixing technologies can be utilized to achieve turbulent mixing- such as the T-mixer and jet mixer. The decision about which system to use can be dependent upon company capacity and needs, material/pump availability, and batch size. However, bridging studies can be done to successfully use these different technologies at different scales to transfer from small-scale up to manufacturing scale.

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About us

ReciBioPharm, a division of Recipharm, is a contract development and manufacturing organization (CDMO) specifically established to focus on serving companies seeking to develop and commercialize advanced therapy medicinal products (ATMPs). ReciBioPharm's specialized CDMO capabilities include pre-clinical to clinical and commercial development and manufacture for new biological modalities encompassing technologies based on live viruses and viral vectors, live-microbial biopharmaceutical products, nucleic acid-based mRNA and plasmid DNA production. Led by a management team and technical experts with a proven track record in both process development and contract manufacturing, ReciBioPharm offers the knowledge and resources necessary to help customers develop and manufacture promising new therapies to meet the needs of patients across the world.