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Considerations for Advancing a Lipid Nanoparticle Formulation to Clinical and Commercial Manufacturing

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A variety of lipid-based systems can be used for delivery of nucleic acids including mRNA and plasmid DNA for both therapeutic and vaccination purposes. Owing to the success of COVID-19 vaccines, lipid nanoparticles (LNPs) have gained substantial interest as a robust and scalable non-viral gene delivery system.

The structure of an LNP consists of four different categories of lipids, which are essential excipients in the formulation of oligonucleotide-based therapeutics and vaccines (Figure 1). Ionizable lipids are responsible for encapsulation of the nucleic acids and play a role in the release of the payload. Polyethylene glycol (PEG) lipids contribute to the stability of the particle and extend the circulation time. Cholesterol and helper lipids provide structural support to the particle and create the necessary rigidity.

Selection of the right lipids with the appropriate quality impacts both the final LNP drug product as well as the LNP process. This white paper provides an overview of critical quality considerations for lipids and describes the process requirements for successful commercial-scale manufacturing.

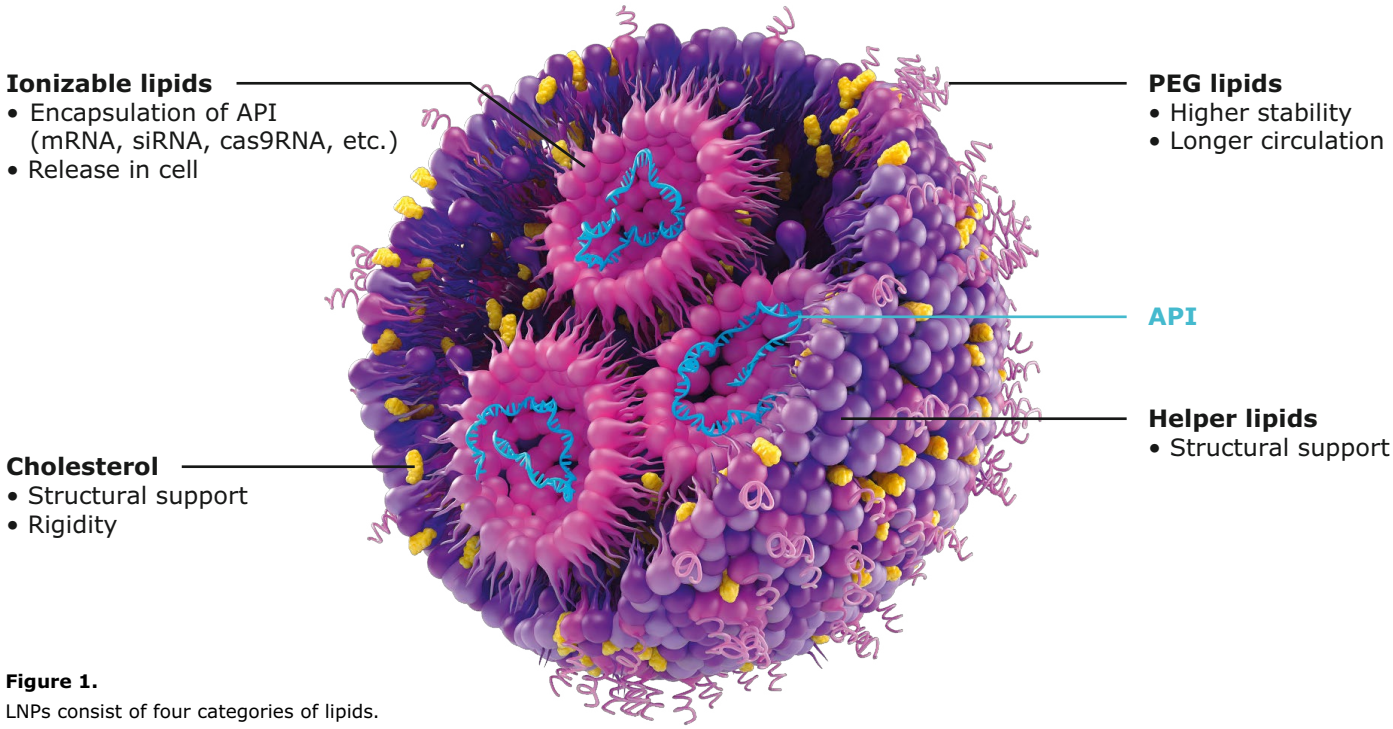


Figure 1.
LNPs consist of four categories of lipids.

Lipid Quality Considerations

Given their central role in the structure and function of the LNP, lipids must meet stringent quality and regulatory requirements. Among the parameters used to define the quality of lipids used in LNP production are purity, consistency, and the overall physicochemical properties (Figure 2):

- **Purity** has a decisive impact on the stability of the overall drug product and the presence of impurities can impact the drug release profile.
- **Consistency** is essential to ensure reproducible results from the drug product as well as in validated processes.
- **Physicochemical properties** are linked to lipid handling properties during the production process and include crystallinity, solubility behavior, stability, and flowability.

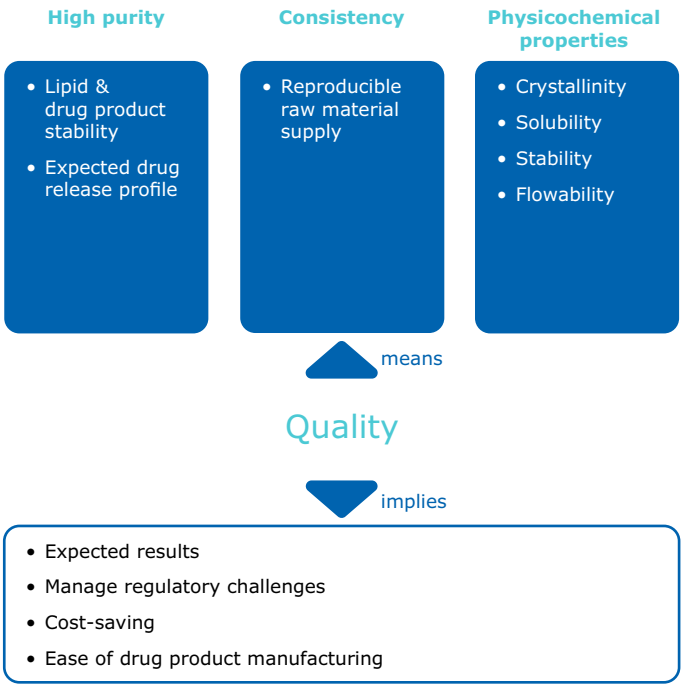


Figure 2.

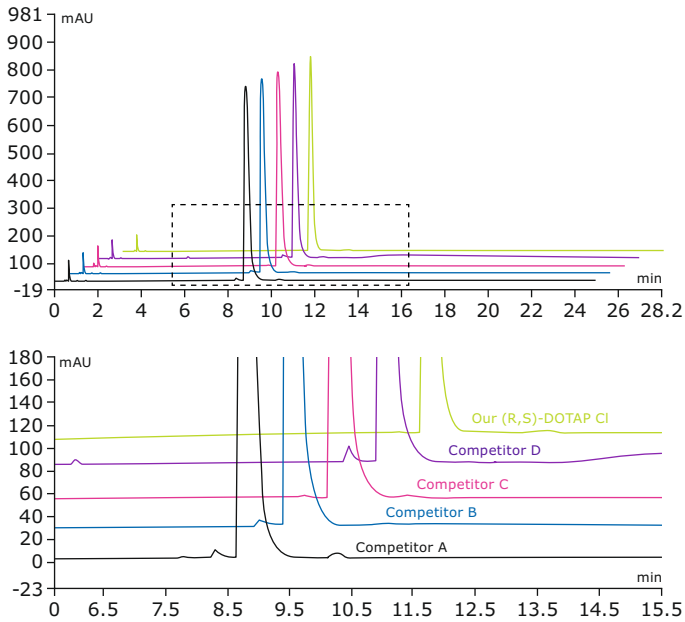
High-quality lipids should be selected to ensure reproducibility, stability, and the desired release profile.

With increasingly stringent regulatory requirements, it is essential to partner with a lipid supplier that can provide both the quality and supporting documentation needed to meet evolving guidelines.

The following studies compared the purity and physicochemical properties of lipids used in LNP production and revealed a wide range of purity across different suppliers. Figure 3 shows a comparison of commercially available cationic (R,S)-DOTAP chloride, a lipid used for encapsulation and delivery of nucleic acid. It is important to use the highest purity lipids as the presence of undesired impurities can have an impact on process performance. Purity is linked directly to LNP stability and impacts the release profile of the drug product.

Good handling properties of lipids are also essential for the formulation process. Figure 4 shows the x-ray powder diffraction (XRPD) spectra of our (R,S)-DOTAP chloride indicating a crystalline powder which facilitates handling.

A well-defined XRPD spectrum indicates that the material has a ordered crystal structure, which typically signifies superior quality when compared to material that produces broad XRPD signal.



Product	Purity, area-%	Largest impurity, area-%
Competitor A	98.2	0.6
Competitor B	98.8	0.3
Competitor C	99.2	0.2
Competitor D	98.5	0.7
Our (R,S)-DOTAP Cl	**99.9**	**≤ 0.05**

Figure 3.

Comparison of the purity of commercially available cationic (R,S)-DOTAP chloride, a lipid used for encapsulation and delivery of nucleic acid.

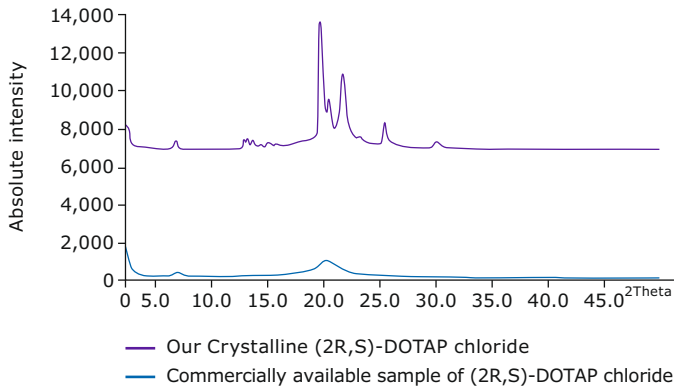
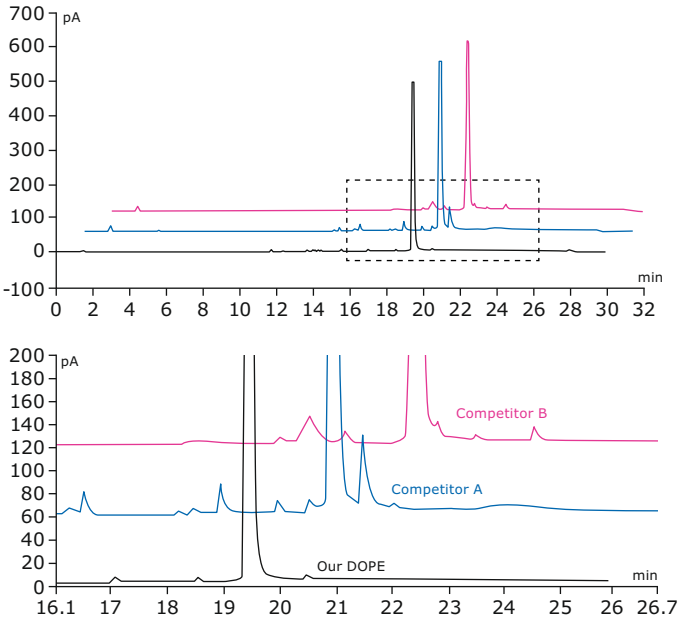


Figure 4.

Comparison of XRPD data of commercially available (2R-S)-DOTAP chloride vs our crystalline (2R,S)-DOTAP chloride.

Figure 5 compares the purity of DOPE, a helper lipid that enables cell uptake and endosomal release. Similar to the (R,S)-DOTAP chloride studies, our lipid showed higher purity compared to a commercially available product. Among other factors, a major contributor to the higher purity for both DOPE and (R,S)-DOTAP chloride excipients is our use of a unique processing and isolation technique which enables different and various purification steps throughout the process to maintain high levels in purity.



Product	Purity, area-%	Largest impurity, area-%
Our DOPE	**93.9**	**1.2**
Competitor A	78.6	3.1
Competitor B	82.2	2.1

Figure 5.

Comparison of the purity of commercially available helper lipid DOPE, used to facilitate cell uptake and endosomal release.

Process Development and Scale-up of LNP Production

Lipids play an essential role in the structural and stability characteristics of LNPs and their quality is critical for the LNP formulation process. The process development of nanoparticles is typically focused on formulation composition, solubilities, stabilities of formulation ingredients, shear sensitivity of API and LNPs, in-process and final product volumes including various process controls. These factors primarily define the process scope into broad categorization of various individual unit operations.

The manufacturing steps for LNP formulations can be broadly categorized into four individual unit operations (Figure 6). The first step is inline mixing of the aqueous API and ethanolic lipids through a suitably designed mixer to produce LNPs. A variety of custom and off-the-shelf mixers and pump technologies can be used and optimized to produce LNPs with the desired characteristics. Among the considerations for design of a controlled process are lipid and API concentrations, dilutions of the streams, dilution buffers, flow rates, pressure, and temperature.

The stream from the mixing step is diluted and collected as a pre-bulk LNP material. This material is concentrated and purified using tangential flow filtration (TFF) consisting of ultrafiltration and diafiltration steps. The diafiltration step enables buffer exchange to a neutral buffer while the ultra-filtration step concentrates the material to a target concentration level. The material is then diluted with a cryoprotectant buffer and sterile filtered by pushing the material through a 0.2 µm membrane to obtain sterile bulk material. The product is subsequently filled into a pre-selected vial size with a pre-determined fill volume based on the dosage and administration requirements in the clinic.

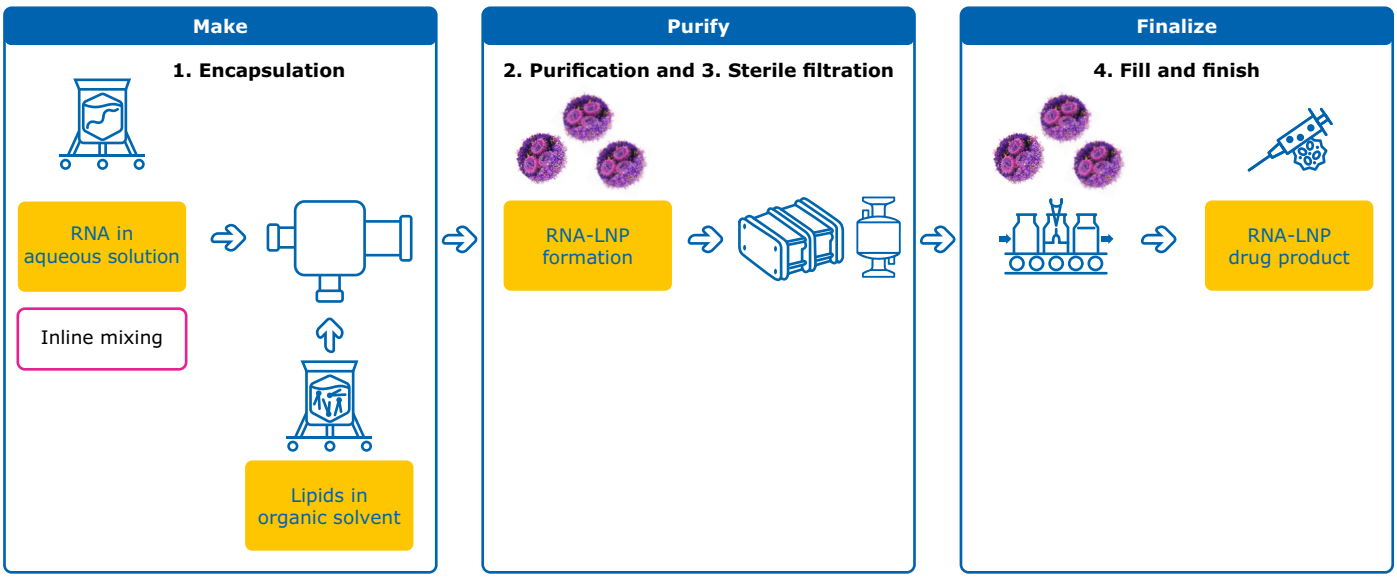


Figure 6.

The LNP production workflow.

Process Development and Optimization

A process which is scalable, controlled, stable and a robust one which delivers consistent product quality is what is typically targeted during the process development. Table 1 summarizes several process parameters which can be incorporated into design of experiment (DoE) and/or optimization studies to tune the physico-chemical characteristics of the nanoparticles to ensure consistent product quality. Changes to the lipid to API ratio (LAR), flow rates of API and lipid feed streams, and injection ratios can be modified to create particles with desired quality attributes. Formulation stability can be further optimized by changing buffer type, pH, osmolality, and the cryoprotectant buffer. The robustness of these formulations, as well as the process, can then be rapidly assessed via stability studies to verify long-term stability of the product.

Process optimization
Lipid & API Concentrations • Lipid Molar Composition (Ionizable Lipids, Cholesterols, Phospholipids, PEG Lipids) • Lipid to API ratio (LAR) **Flow-Rates** **Injection Ratios (A/O)**
Formulation optimization
Buffer Type **Salt Composition** **pH** **Osmolality/Strength** **Cryoprotectant**
Stability
In-Process Stability **Conventional Stability** • Stress-induced • Accelerated • Normal **Freeze-Thaw Studies**

Table 1.

Several process parameters offer the ability to tune physiochemical characteristics of LNPs.

There are different ways of optimizing LNPs. The following case study describes the process development of an RNA-based LNP generated via turbulent mixing. The lipid concentration, flow rates, and injection ratios were evaluated in terms of their impact on the LNP size, polydispersity index (PDI) and encapsulation.

LNP size and PDI were impacted by varying lipid concentration, flow rates, and injection ratios. An increase in lipid concentration coupled with different flow rates affected the particle size; the size of the spread became tighter with an increase in lipid concentration. With respect to flow rate, there was an inverse trend where high flow rates led to particles of lower size. Higher injection ratios of the aqueous and ethanolic lipid streams led to particles of larger size; when flow rates and the lipid concentrations were similar, a high injection ratio led to particles of larger size versus low injection ratios.

The PDI decreased with increased lipid concentration. The spread in PDI was minimized with increased lipid concentration. With similar flow rates and lipid concentrations, a higher injection ratio led to a higher PDI versus a lower injection ratio which led to lower PDI.

Injection ratios also affect encapsulation efficiency. In general, an increase in lipid concentration coupled with low flow rates led to higher encapsulation efficiency. In data sets where the lipid concentration and flow rates were similar, the injection ratio had the ability to impact the encapsulation efficiency (data not shown). Similarly, higher flow rates and low injection ratios related to high encapsulation efficiency while the high injection ratio resulted in lower encapsulation efficiency.

It should be noted that the trends observed in these studies are specific to the formulation and the LNP mixer developed and designed for this process. It is likely that these trends would change with a different formulation or mixing design.

LNP Downstream Processing

Downstream processing steps, which include homogenization, extrusion, TFF, and clarification, ensure that the nanoparticles are homogenous and stable during storage and are free of residual contaminants.

The extrusion step reduces particle size and generates uniform particle size distributions (decreases PDI) and involves pushing the bulk nanoparticles via pressure through membranes whose pores can range from fifty to hundreds of nanometers. This step is generally performed with liposomal-based ethanolic injection processes. TFF can be optimized in a number of ways including the membrane material, its molecular weight cutoff, inlet cross-flow rate (L/min/m^2), permeate flux (LMH), product membrane loading (kg/m^2), transmembrane pressure (psi), surface area, and the cryoprotectant buffer. Similarly, the clarification and sterile filtration steps, essential for sterility assurance, can be optimized based on the membrane type, pore size (for dual membranes) and construction, surface area, and pressures.

The fill/finish step involves the filling of drug product into a pre-selected vial size and with a pre-determined fill volume based on the dosage and administration requirements in the clinic. The vials are inspected, labelled and right away taken into cold storage (-20 °C or -80 °C). Alternatively, post-filling, the vials are lyophilized.

LNP Process Scale-up and GMP Tech Transfer

Scale up is performed as a factor of time where the process is allowed to run until the API and/or lipid stock solutions are consumed. The mixer internal diameter, flow rates, tubing diameters, and length of the repeat tubing are all typically fixed when scaling to execute a tech transfer of a like-for-like process developed in the lab into GMP. Stability of the material in terms of hold times spanning the mixing and processing times should be assessed as the product will be at room temperature in low pH buffer. In terms of process controls, in-process testing, temperature and pressures should be assessed to determine whether critical operation steps need to be gated and identify process hold times based on the duration and dynamics of how the process runs in the GMP setting.

LNP Analytical Development

Analytical methods are essential to monitor and confirm critical quality attributes. Among the critical quality attributes (CQAs) for an LNP formulation are particle size, PDI, API encapsulation efficiency, concentration, and lipids content, and impurities. Other tests include residual ethanol/solvents, API purity and sterility/bioburden and endotoxin.

Conclusion

The choice of lipids has a strong impact not only on the LNP product, but also on the LNP process. Characteristics such as purity and physico-chemical properties must be considered.

Efficacy of LNP-based vaccines and therapeutics relies, mostly, on the lipid composition of the LNP, which varies from product to product. A small change in the relative abundance of a particular lipid could have a significant impact on how that drug is delivered and its efficacy.

Process development of LNPs identifies and defines appropriate process controls and critical process parameters during each unit operation while maintaining the CQAs of the LNPs to enable a seamless scale up. The established process parameters should be considered and readily available when mapping the scale up and tech transfer activities. These process parameters, in combination with the right mixer, offer the ability to tune the physico-chemical characteristics of the LNPs during process development. Choosing an appropriate and scalable mixing technology is essential to enable successful and expeditious clinical and commercial manufacturing of LNPs.

Lipid quality and formulation/process development expertise and capabilities should therefore be decisive factors for both lipid supply and process development of LNPs when selecting a partner for development and clinical and commercial manufacturing of LNPs for vaccine and therapeutic applications.

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