c&en | WHITE PAPERS

HARNESSING CHEMISTRY FOR SCALABLE MANUFACTURE OF LIPIDS USED IN MRNA DELIVERY

A global rollout of effective vaccines was crucial in ending the COVID-19 pandemic; it's estimated that as many as 19 million deaths per year were prevented.[1,2] In the US and European Union, more than 90% of those vaccines relied on lipid nanoparticle (LNP) technology to deliver messenger RNA (mRNA).[3] LNP-mRNA vaccines could be rapidly designed and deployed, and the public health emergency created a launchpad for maturing and validating LNP delivery technology. These vaccine programs also demonstrated that LNP formulations can be prepared at the massive scale required for the global vaccination programs.

The US Food and Drug Administration issued its first approval for an LNP as a drug delivery vehicle in 2018, for patisiran. This medication uses short interfering RNA (siRNA) to silence a gene involved in transthyretin amyloidosis, a rare neurodegenerative disease. Now, with abundant evidence of efficacy and safety after the rollout of SARS-CoV-2 vaccines, LNPs are being trialed as therapeutic delivery agents for a range of conditions, including cystic fibrosis,[4] Alzheimer's disease,[5] and several cancers.[4]

Demand for LNP therapeutics has been increasing because of their successes during the COVID-19 pandemic and their potential as drug delivery systems. However, the large-scale production of LNPs presents a significant process chemistry and purification challenge, particularly for the main component of LNPs: lipids. Read on to learn how lipid synthesis and purification can be optimized to enable new generations of LNP-based therapies.

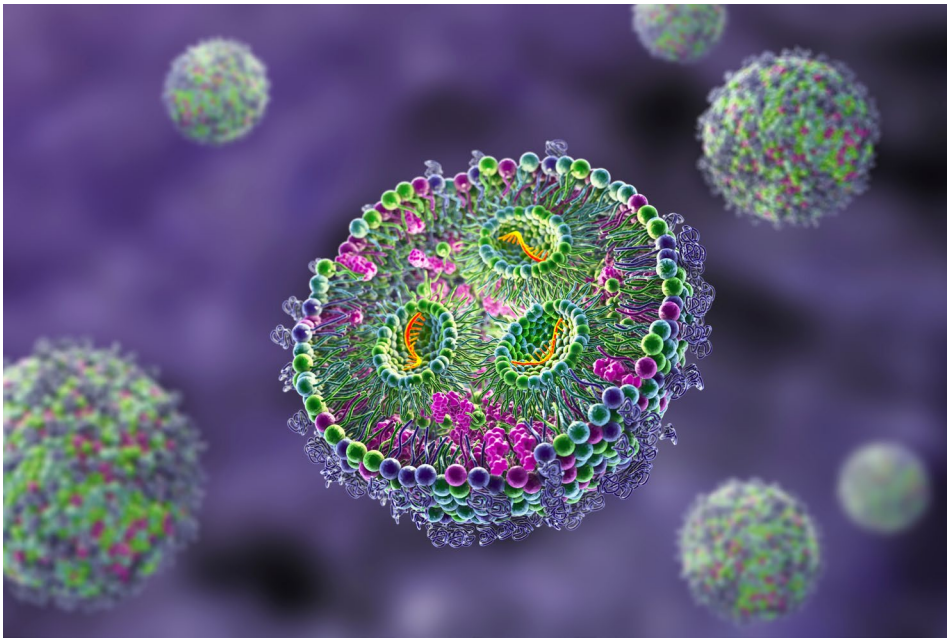

Figure 1. Lipid nanoparticle mRNA vaccines played a critical role in ending the COVID-19 pandemic.

Credit: Kateryna Kon/Shutterstock.com

THE ANATOMY OF A LIPID NANOPARTICLE

LNPs are typically formulated with four key components: phospholipids, ionizable cationic lipids, cholesterol, and polyethylene glycol–linked (PEGylated) lipids (see Box). Like the lipids that make up every cell membrane, LNPs encase and protect their cargo. Easily degraded payloads, like mRNA, are protected from degradation until the LNP can deliver its contents to cells. LNPs are typically spherical, with an average diameter between 10 and 1,000 nm, and encased material can include nucleic acids, protein fragments, or other biological payloads.

Significant efforts have gone into engineering LNP components to be compatible with nucleic acid cargo. Nucleic acids carry a polyanionic charge, which makes them repel negatively charged phospholipids. The development of ionizable cationic lipids was crucial for mRNA-LNP vaccines. These lipids, which hold a positive charge at acidic pH, surround and encase nucleic acids during storage. Once LNPs are injected and enter a pH-neutral bloodstream, the ionizable lipids regain neutrality, which helps the LNPs evade immune detection. Both particle hydrophobicity and positive charge have been associated with increased immunological response.[6,7] The LNPs are taken up into cells through endocytosis, but they become sequestered in endosomes, organelles destined for downstream destruction. The ionizable lipids then regain their positive charge in the acidic environment of endosomes, which ultimately disrupts the LNP structure and releases nucleic acids within the cell.[8]

A TYPICAL MESSENGER RNA–LIPID NANOPARTICLE (MRNA-LNP) VACCINE CONTAINS FIVE KEY COMPONENTS:

mRNA. The cargo. In the Pfizer/BioNTech SARS-CoV-2 vaccine, this sequence is 4,250 nucleotides long.[9]

Ionizable cationic lipid: Approximately 60% of the LNP by weight.[10] Its positive charge binds to the negatively charged mRNA. Adopting a neutral charge in blood, it evades the immune system and can circulate and access target cells. Inside the acidic endosomes in cells, its positive charge is re-established. This triggers LNP disruption and cytosolic mRNA release.

Cholesterol: Approximately 25% of the LNP by weight. Promotes fusion of the LNP surface with cell membranes and limits plasma protein interactions. Improves bilayer stability and rigidity.

Phospholipid: Approximately 10% of the LNP by weight. Supports and promotes cell binding. Increases bilayer structural stability.

PEGylated lipid: Approximately 5% of the LNP by weight. Important in LNP size control, particle stability, and particle aggregation control.
Aids in extending systemic circulation.

Both the Comirnaty® (Pfizer/BioNTech) and Spikevax® (Moderna) SARS-CoV-2 vaccines contain the four LNP non–nucleic acid components in similar molar ratios. However, they differ in the ionizable lipid and PEGylated lipid, though these components are relatively similar (Fig. 2). While the specific structures may vary, these lipids must be synthesized at high purity standards to ensure the safety of LNP-based therapeutics.

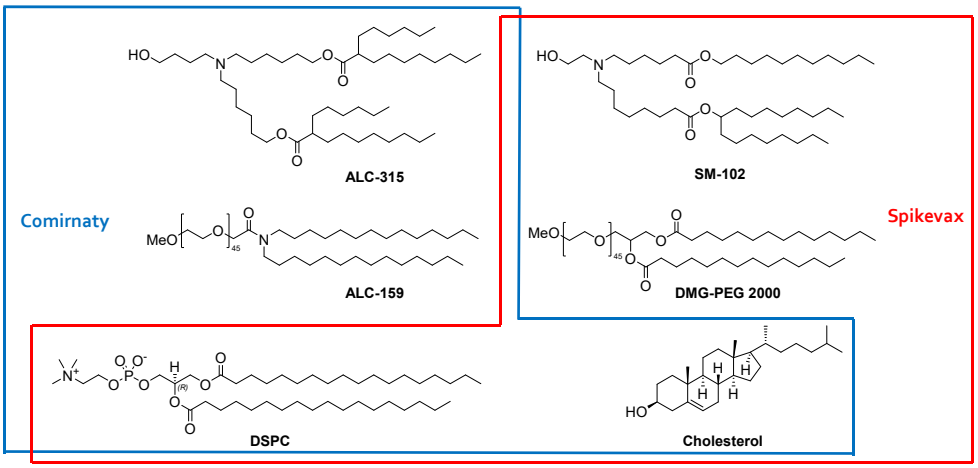

Figure 2. Several lipid components make up LNPs.[11]

Credit: Curia Global, Inc.

CLEAN CHEMISTRY IS KEY

Synthesizing lipids is relatively straightforward and uses well-known reactions such as esterification, amination, alkylation, and protecting group removal. However, side reactions can produce particularly "nasty" by-products that are difficult to remove later, according to Joerg Jung, senior director of chemical development at Curia.

Lipid synthesis involves intermediates and final products that are typically oils that cannot be purified by crystallization or distillation. Separation by chromatography is often the only feasible option. However, chromatography is often the most expensive step of lipid production. These costs are amplified as production scales or timelines are compressed. Lipid products are typically purified only at the final stage of their synthesis.

"The fate of each side component needs to be evaluated in all steps of the downstream chemistry," says Jung. "This is required to avoid the presence of impurities that coelute with the desired lipid component in the chromatography."

Achieving clean chemical syntheses relies on several conditions.

- The choice and quality of starting materials and reagents is fundamental.
- Reactions should proceed with good conversion and a simple impurity profile, although yield can be reduced in favor of an easier purification in some cases.
- Highly reactive intermediates should be avoided, as they are more likely to create byproducts.
- When byproducts are formed, for example, from coupling reagents, it is ideal if they can be removed with simple aqueous washes.
- Careful evaluation of the impurity profile can reveal the importance of reagent stoichiometries.

Ultimately, understanding the sources of impurities and how they accumulate in subsequent reactions or interfere with purification is crucial. "Good chemical development is key to reducing purification costs and compressing timelines," Jung says.

THE CHALLENGE OF CHROMATOGRAPHY

Though different types of liquid chromatography are helpful in the purification of the lipids used in LNPs, normal-phase chromatography (NPC) is preferred for scaling a lipid synthesis. "Everyone prefers normal-phase chromatography," says José-Luis Barredo, general manager and head of global fermentation at Curia. Its counterpart, reverse-phase chromatography, uses more expensive resins, and material to be loaded onto columns must be dissolved in aqueous solution (as an example, lipids are not soluble in water).

NPC uses a polar stationary phase (often silica gel) and a nonpolar mobile phase to separate polar compounds according to differences in polarity. Synthetic impurities that arise during manufacturing must elute significantly before or after lipid products, and it is ideal if the maximum fraction of the lipid elutes near the midpoint of the column's elution profile, Barredo explains.

Though solvents, mobile phase gradients, and flow rates can all be optimized to establish this peak separation, the most successful separations will start with low amounts of impurities in the crude product. According to Barredo, lipids typically must be at least 98–99% pure for use in LNPs. He added that achieving this standard with a single chromatographic separation is impossible when the crude reaction mixture is 50% pure or less. The chemistry has to be optimized to reach greater purity (usually about 75%), after which one chromatographic separation is often sufficient.

Scaling the purification also presents challenges. "One of the main challenges when you scale, for example, from 50-liter columns to 500-liter columns is to confirm that the silica gel (or the resin) doesn't collapse, that the flow rates and working pressure are scalable, et cetera," Barredo explains.

Parameters that need to be optimized when scaling the purification include:

- mobile-phase polarity
- column loading
- column height versus diameter
- flow rate
- pressure
- temperature

In addition to careful optimization, large-scale chromatographic separations require specialized technology. This equipment includes solvent reservoirs connected to fluid pumps through in-line flowmeters, static mixers, switching valves, and chromatography columns (glass or stainless steel).

Curia, which supported the manufacture of lipids for SARS-CoV-2 vaccines during the COVID-19 pandemic, has the expertise and equipment needed to navigate these scalability challenges. "Curia researchers' work enabled vaccine doses to reach patients," Barredo says.

Figure 3. Curia 500 L chromatography columns used for lipid purification

Credit: Curia Global, Inc.

WHAT'S NEXT FOR LNPS?

Both the Pfizer and Moderna SARS-CoV-2 vaccines need to be stored at temperatures as low as –80°C, necessitating a complex cold-storage supply chain. More-temperature-stable LNPs would enable the delivery and administration of these vaccines in places where the supply chain is not yet in place. Better stability would also simplify delivery and storage logistics at all locations.

One promising avenue for improving the thermal stability of LNPs is optimizing the structure and ratio of the lipids that comprise the LNP. In a recent example, piperidine-based ionizable lipids were used to create stable mRNA-encasing LNPs under simple refrigeration as liquid preparations.[12] This approach works because it avoids using tertiary amines, which can form aldehyde impurities that can then react with mRNA nucleosides. Interestingly, patisiran, with similar tertiary amine–containing ionizable lipids, is stable under normal refrigeration, but apparently this is due to the greater stability of siRNA compared to that of mRNA.[13] Similar lipid changes may also improve the cytosolic release of nucleic acids and aid vaccine efficacy. Additional measures that may improve thermal stability include the addition of stabilizing additives and LNP lyophilization.

Another avenue for future improvement is targeted delivery. LNPs administered intravenously easily access the liver. However, they "can optionally have embedded targeting molecules such as antibodies, cell-targeting peptides, and/or other drug molecules at the exterior surface," Barredo says. "This opens a wide future for LNPs," because they can be modified to reach a specific tissue or cell type. Adding unique lipids can also direct the LNPs to specific organs.[14]

Tissue targeting creates an opportunity to improve the specificity and efficacy of LNPs. Research is ongoing to determine how best to link LNPs with antibodies, peptides, or other tissue-targeting functionalization.[15,16] Efforts here are challenged by the need to maintain the ability of modified LNPs to protect and deliver their cargo. The addition of antibodies or other biological species will also significantly complicate the synthesis and purification of lipids or LNPs.

NAVIGATING THE CHALLENGES OF LIPID MANUFACTURING WITH EXPERTISE

As LNP-based therapeutics move out of preliminary research and into testing, their lipid components will require scaled-up synthesis and purification. A partner familiar with the challenges of lipid manufacturing can help bring these new technologies to the clinic sooner. Curia scientists and engineers have successfully supported LNP development and production for SARS-CoV-2 vaccines and a range of additional programs. Curia's partners trust their expertise in chemical synthesis, chromatography, and analytical testing to support their entire pipeline of LNP development and production.

REFERENCES

1. Craig Mellis, ed. "Lives Saved by COVID-19 Vaccines." *J. Paediatr. Child Health*, 58, no. 12 (Nov. 2022): 2129. https://doi.org/10.1111/jpc.16213.
2. Oliver J. Watson et al., "Global Impact of the First Year of COVID-19 Vaccination: A Mathematical Modelling Study." *Lancet Infect. Dis*. 22, no. 9 (Sept. 2022): 1293–1302. https://doi.org/10.1016/S1473-3099(22)00320-6.
3. "COVID-19 vaccine doses administered by manufacturer." Our World in Data. Accessed Oct. 3, 2024. https://ourworldindata.org/grapher/covid-vaccine-doses-by-manufacturer?country=OWID_EU27~USA.
4. Yeung Wu, Sinuo Yu, and Irene de Lázaro, "Advances in Lipid Nanoparticle mRNA Therapeutics beyond COVID-19 Vaccines." *Nanoscale* 16, no. 14 (April 4, 2024): 6820–36. https://doi.org/10.1039/d4nr00019f.
5. Mungyo Jung et al., "A Therapeutic Nanovaccine that Generates Anti-Amyloid Antibodies and Amyloid-specific Regulatory T Cells for Alzheimer's Disease." *Adv. Mater*. 35 no. 3 (Jan. 19, 2023): 2207719. https://doi.org/10.1002/adma.202207719.
6. Yuanchang Liu et al., "Effects of Engineered Nanoparticles on the Innate Immune System." *Semin. Immunol*. 34 (Dec. 2017): 25–32. https://doi.org/10.1016/j.smim.2017.09.011.
7. Daniel F. Moyano et al., "Nanoparticle Hydrophobicity Dictates Immune Response." *J. Am. Chem. Soc*. 134, no. 9 (March 7, 2012): 3965–7. https://doi.org/10.1021/ja2108905.
8. Xuexiang Han et al., "An Ionizable Lipid Toolbox for RNA Delivery." *Nat. Comm*. 12, no. 1 (Dec. 2021): 7233. https://doi.org/10.1038/s41467-021-27493-0.
9. Jacques Demongeot and Cécile Fougère, "mRNA COVID-19 Vaccines—Facts and Hypotheses on Fragmentation and Encapsulation." *Vaccines* 11, no. 1 (Dec. 24, 2022): 40. https://doi.org/10.3390/vaccines11010040.
10. Barnabas Wilson and Kannoth Mukundan Geetha, "Lipid Nanoparticles in the Development of mRNA Vaccines for COVID-19." *J. Drug Delivery Sci. Technol*. 74 (Aug. 2022):103553. https://doi.org/10.1016/j.jddst.2022.103553.
11. Yuta Suzuki and Hiroshi Ishihara, "Difference in the Lipid Nanoparticle Technology Employed in Three Approved siRNA (Patisiran) and mRNA (COVID-19 Vaccine) Drugs." *Drug Metab*. Pharmacokinet. 41 (Dec. 2021): 100424. https://doi.org/10.1016/j.dmpk.2021.100424.
12. Kazuki Hashiba et al., "Overcoming Thermostability Challenges in mRNA–Lipid Nanoparticle Systems with Piperidine-Based Ionizable Lipids." *Communications Biology* 7 (2024): 556. https://doi.org/10.1038/s42003-024-06235-0.
13. Linde Schoenmaker et al., "mRNA-Lipid Nanoparticle COVID-19 Vaccines: Structure and Stability." *Int. J. Pharm*. 601 (May 2021): 120586. https://doi.org/10.1016/j.ijpharm.2021.120586.

14. Xu Wang et al., "Preparation of Selective Organ-Targeting (SORT) Lipid Nanoparticles (LNPs) Using Multiple Technical Methods for Tissue-Specific mRNA Delivery." *Nat. Protoc.* 18, no. 1 (Jan. 2023): 265–91. https://doi.org/10.1038/s41596-022-00755-x.
15. Dong-yup Lee et al., "Strategies for Targeted Gene Delivery Using Lipid Nanoparticles and Cell-Derived Nanovesicles." *Nanoscale Adv.* 5, no. 15 (July 7, 2023): 3834–56. https://doi.org/10.1039/d3na00198a.
16. Hidefumi Mukai, et al., "Recent Advances in Lipid Nanoparticles for Delivery of Nucleic Acid, mRNA, and Gene Editing-Based Therapeutics." *Drug Metab. Pharmacokinet.* 44 (June 2022): 100450. https://doi.org/10.1016/j.dmpk.2022.100450.