A Deeper Look at Lipid Nanoparticles

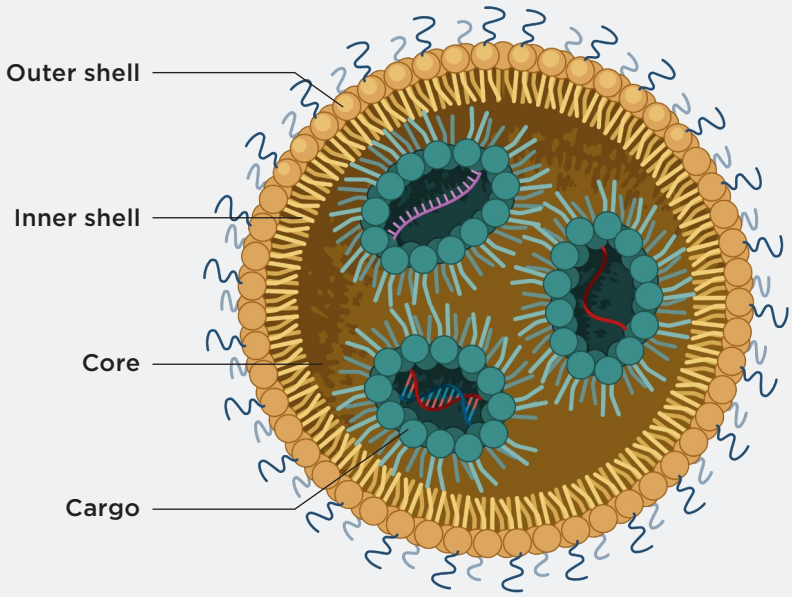
Anis Larbi, PhD, Medical and Scientific Affairs, Beckman Coulter Life Sciences

1. Introduction

Small particles, often referred to as nanoparticles upon the nanometer scale, play a significant role in various scientific fields. These spherical particles, which typically measure between 50 nanometers to 1000 nanometers, exhibit unique physical and chemical properties due to their high surface area to volume ratio and quantum effects.[1] In environmental science, small particles can be pollutants, affecting air and water quality, as well as posing health risks when inhaled. In medicine, lipid nanoparticles (LNPs) are used for drug delivery and therapeutic agents due to their ability to interact with biological systems at the cellular level.[2] Additionally, in materials science, they enhance the strength, durability, and functionality of materials, leading to innovative applications in electronics, coatings, and energy storage. The study and manipulation of small particles continue to drive advancements across multiple disciplines, highlighting their importance as an emerging technology. Here we focus on understanding lipid nanoparticles and their use within the biomedical realm.

LNPs are optimized for RNA delivery and scalable for manufacturing since they are entirely synthesized in laboratories, exhibiting an enhanced circulation stability.[3] This is a significant advantage over extracellular vesicles which are more difficult for cargo loading control due to their natural origin for upscaling manufacturing processes and characterizations. Prepared by micro emulsification, sonication, or high-pressure homogenization, LNPs have emerged as the most successful gene delivery systems, particularly highlighted by their use in COVID-19 vaccines.[4]

LNPs are multicomponent lipid structures that typically include a phospholipid, an ionizable lipid, cholesterol, and a PEGylated lipid. The structure of an LNP is defined by three main components: the core, inner shell, and outer shell (**Figure 1**). The outer shell consists of a hydrated PEGylated layer, while the inner shell contains cholesterol, phospholipids, and cationic ionizable lipids. The core features a distorted hexagonal or worm-like phase comprising cationic ionizable lipids, cholesterol, and RNA intended for delivery. LNPs have only one phospholipid outer layer that encapsulates the interior while liposomes have one or more rings of lipid



Advantages:

- Market approved
- Optimized for RNA delivery
- Scalable manufacturing
- Enhanced circulation stability

Limitations:

- Possible immune reactions
- Toxicity
- Off-target effect
- Size constraints for cargo
- Inconsistent body distribution

Figure 1. General structure of a lipid nanoparticle

bilayer surrounding an aqueous pocket. RNA encapsulation is achieved by mixing an ethanolic solution of lipids and sterols with an acidic aqueous RNA solution which is then treated to match physiological compatibility. In summary, LNPs are biocompatible and biodegradable ingredients demonstrating high cellular uptake, good acidic pH drug protection, long shelf life, and ease of drug entrapment. For these reasons, despite any limitations (undesired immune reaction), several LNP-based therapies have already been approved (Patisiran [siRNA-LNP], BNT162b2 [Pfizer/BioNTech mRNA COVID-19 vaccine], mRNA-1273 [Moderna mRNA COVID-19 vaccine], mRNA-1345 [Moderna RSV vaccine], ARCT-154 [Arcturus/CSL saRNA COVID-19 vaccine], and Tobramycin [chronic pulmonary infection]).

2. LNP manufacturing

Numerous methods are available for the synthesis of LNPs. In addition to choosing lipid types, their ratios and concentrations for their formulation, the method chosen determines the attributes of the end-product (particle size, encapsulation efficiency and yield, and polydispersity index). These properties are crucial for determining pharmacokinetics, cellular uptake, and the overall drug efficacy, toxicity, and biodistribution.[5] The manufacturing of LNPs can be performed using different methods:

- **Top-down methods:** Methods applying energy to break larger particles into smaller ones (also known as high-energy methods). This technique involves high-pressure homogenization (500 to 5000 bar) which can generate high concentrations of LNPs or ultrasonic homogenization (ultrasound waves will create cavitation) with a low encapsulation efficiency drawback.
- **Bottom-up methods:** These methods depend on lipid components merging to create uniform nanoparticle systems. The Bangham method is the historical technique used for LNP and liposome formulation. By dissolving lipids in organic solvents, a thin lipid film is evaporated with solvents and hydrated with an aqueous solution to trigger nanoparticle formation.

- **Nanoprecipitation-based methods:** Self-assembly is the primary approach with this method which was used in the COVID-19 vaccine. An aqueous phase containing the oligonucleotide is mixed with a water-miscible solvent containing the lipids. The overall drop in lipid solubility triggers self-assembly and growth to create nanoparticles.
- **LNP synthesis using microfluidics:** Finely controlling mixing parameters to obtain homogenous LNP populations and good batch-to-batch reproducibility by nanoprecipitation is extremely important. The approach for this method works by controlling the flow rate, efficiency, repeatability, and encapsulation efficiency, thus rendering this technique as the current state-of-the-art LNP formulation method.

Choosing the right formulation process is a crucial decision that shapes the entire drug development journey. An optimized formulation method ensures the production of high-quality LNPs and reduces the risks linked to suboptimal formulations, such as inconsistent performance or issues during scale-up. Achieving consistency and reproducibility with different scales is essential for meeting regulatory standards and successful clinical applications, making the formulation process a key element of effective drug development.

For example, the initial steps of the RNA delivery process involve testing numerous RNA/LNP complexes (potentially several hundred) with varying RNA, lipid mixtures, and synthesis conditions to identify the most optimal combination (**Figure 2**). Due to the high cost of lipids and RNA, working with very small volumes is standard practice during this stage. Throughout the entire drug mass production process, it's crucial to maintain the consistency of the RNA/LNP complex at every development stage. As the process progresses towards human trials and production, it is essential to adhere to current Good Manufacturing Practices (GMP) to ensure compliance with pharmaceutical industry guidelines, enabling a smooth and efficient transition of the drug product to market.[6]

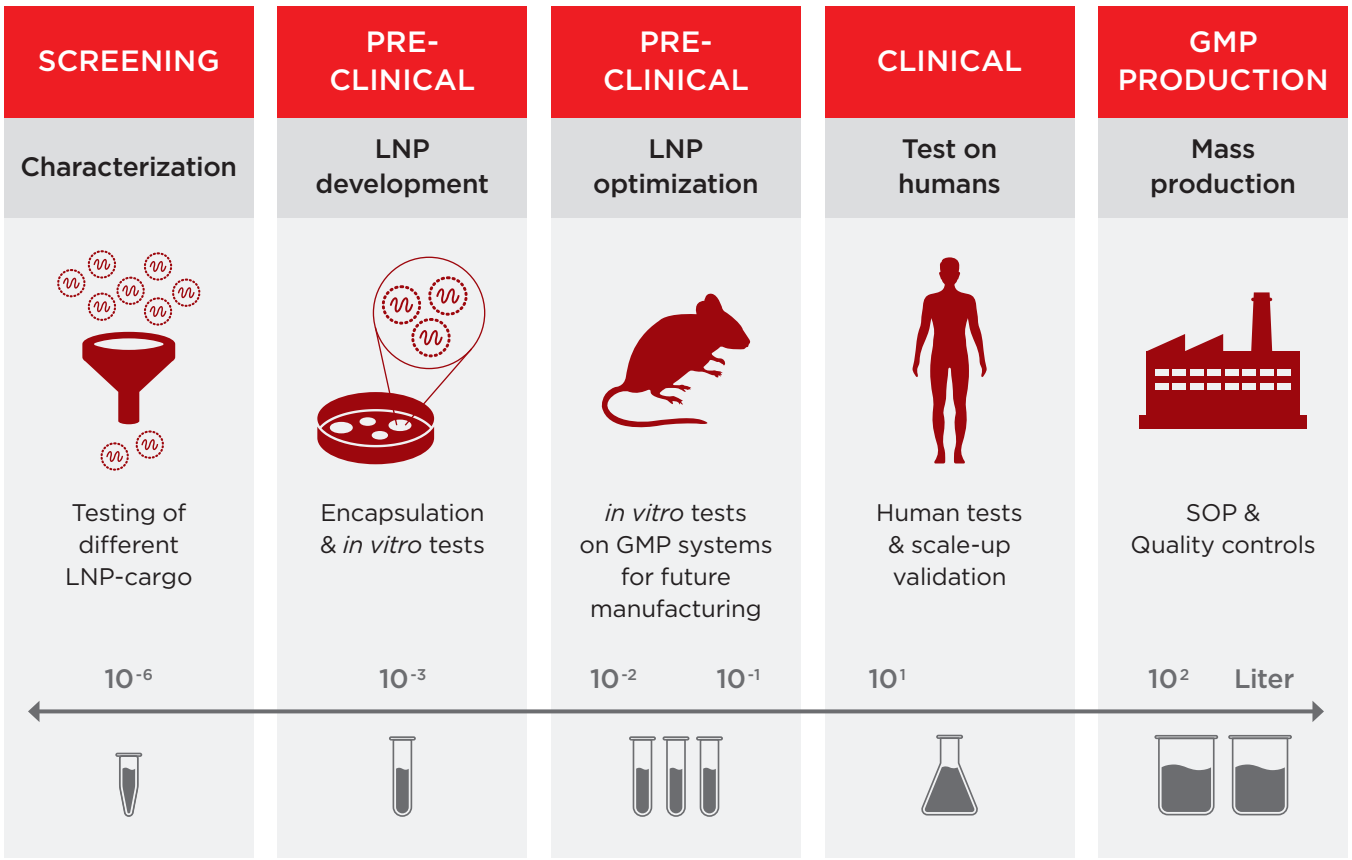


Figure 2. Main steps of LNP manufacturing

3. Optimizing drug efficacy

3.1 The future of drug delivery

In the field of drug delivery, small particles, particularly lipid nanoparticles, have emerged as a groundbreaking technology that enhances the precision, efficacy, and safety of therapeutic treatments. As described above, LNPs can be engineered in many ways to carry drugs directly to targeted cells or tissues, thereby minimizing side effects and improving the therapeutic index. By utilizing various materials (lipids, polymers, and other reagents), LNPs can be designed to release their payload in a controlled manner, responding to specific stimuli such as pH changes, temperature variations, or enzyme presence. This targeted delivery not only maximizes the drug's effectiveness but also reduces the required dosage, leading to fewer adverse reactions.[7] Moreover, nanoparticles can cross biological barriers, such as the blood-brain barrier, which traditional drug delivery systems struggle to penetrate. This capability opens new avenues for treating complex conditions like cancer, neurodegenerative diseases, and infections. The versatility and precision of nanoparticle-based drug delivery systems hold immense promise for advancing personalized medicine and improving patient outcomes. Currently, there are >80 clinical trials using LNPs as vehicles aiming to treat various health conditions such as infections, cardiovascular diseases, dental issues, bone diseases, cancers, gene deficiencies, metabolic diseases, fibrosis conditions, and more. Soon, many of the successful clinical trials will result in increased numbers of marketed LNP-based therapies.

Advantages of lipid nanoparticles for drug delivery

LNPs offer several advantages in clinical trials, including:

- Enhanced stability and protection of encapsulated therapeutics
- Efficient cellular uptake and endosomal escape
- Broad delivery of a wide range of therapeutic agents
- Strong potential for targeted delivery achievement with surface modification
- Effective scalability and feasibility for large-scale production

3.2 Reaching the brain

Worldwide, central nervous system (CNS) diseases, such as Alzheimer's disease, Parkinson's disease, strokes, and brain cancers, are among the leading causes of disability and have consequently garnered significant attention. Despite this recognition, only a few drugs have proven effective in treating these conditions, largely due to numerous challenges. Amongst these hurdles, the primary obstacle relates to the inefficiency of drug transportation across the blood-brain barrier (BBB). The success of drug transport across the BBB is heavily influenced by several factors (molecular size, hydrophilicity, and dissociation degree). Since most small-molecule drugs and nearly all macromolecular drugs (e.g., recombinant proteins, therapeutic antibodies, and nucleic acids) struggle to cross the BBB, developing drug delivery systems which can efficiently transport therapeutic agents into the CNS is crucial for advancing the treatment of CNS diseases.

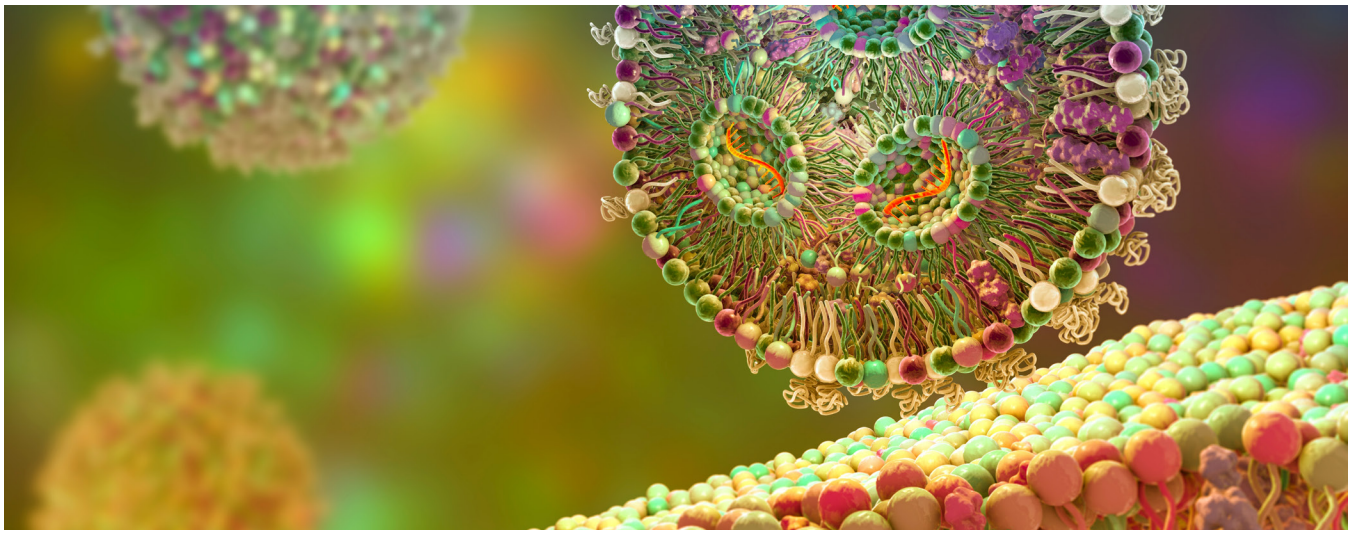
Nanoparticles can be engineered to carry drugs across the BBB by exploiting mechanisms such as receptor-mediated transcytosis.[8] These nanoparticles can be functionalized with ligands that bind to receptors on the BBB, facilitating their transport into the CNS. Pre-clinical models are used for screening and optimization of the LNP configurations to maximize mRNA delivering efficiency into the brain. It is now possible to transfect neurons and astrocytes of the whole entire brain after intravenous injections using LNPs.

3.3 LNPs in CAR-T therapy

The use of LNPs in *in vivo* CAR-T therapy represents a novel and promising approach to enhance the precision and efficacy for cancer as well as for other therapies. Traditionally, chimeric antigen receptor T-cell (CAR-T) therapy involves *ex vivo* modification of a patient's T-cells, which is labor-intensive and costly. However, LNPs offer a groundbreaking alternative by enabling the direct delivery of CAR-encoding mRNA or DNA to T-cells within the patient's body.[9] These nanoparticles can be engineered to target specific T-cell populations, facilitating the in-situ generation of CAR-T cells without the need for extraction and re-infusion. This method leverages the biocompatibility and efficient cellular uptake of LNPs, allowing for rapid and targeted genetic modification. Preclinical studies have demonstrated that LNP-mediated delivery of CAR constructs can induce robust anti-tumor responses, significantly simplifying the manufacturing process and reducing treatment turnaround time. As research advances, the integration of LNPs in CAR-T therapy holds the potential to make this powerful immunotherapy more accessible and scalable, ultimately improving outcomes for patients with various types of cancer.

4. Conclusion

Lipid nanoparticles (LNPs) have emerged as a promising solution for manufacturing the next generation of therapies, potentially improving drug efficiency compared to current delivery methods. The field of LNPs is rapidly expanding and has made significant advancements in recent years. LNPs have proven their value with quickly developing therapies for infectious diseases (mRNA vaccines) and are now being used to treat a variety of health conditions.[10] As interest expands and the variety of LNPs increases, there will be a need for better standardized processes to meet regulatory requirements. One current challenge related to this occurrence is the characterization of LNPs and the measurement of loading efficiency in a standardized, accurate, cost-effective, simplified, and reproducible manner. Ultracentrifugation is a method that has shown promising results in reducing the heterogeneity in size and mRNA loading levels.[11] Along with single-particle characterization, understanding the bulk sample is important to understanding the total heterogeneity and the RNA loading levels, which analytical ultracentrifugation can help achieve.[12] The characterization of small particles such as LNPs is important and requires their analysis at the single-particle level.[13] This could be achieved using flow cytometry, provided the instruments possess the necessary sensitivity, accuracy, and reproducibility qualifications.



5. References

1. Alfutaimani, Alanood S et al. “Exploring the landscape of Lipid Nanoparticles (LNPs): A comprehensive review of LNPs types and biological sources of lipids.” *International journal of pharmaceutics: X*, 2024, 8: 100305.
2. Vasileva, Olga et al. “Composition of lipid nanoparticles for targeted delivery: application to mRNA therapeutics.” *Frontiers in pharmacology*, 2024, 15: 1466337.
3. Let’s talk about lipid nanoparticles. *Nat Rev Mater,* 2021, 6: 99.
4. Trollmann, Marius F W et al. “Revisiting lipid nanoparticle composition and structure: A critical take on simulation approaches.” *Proceedings of the National Academy of Sciences of the United States of America*, 2025, 122,9: e2422995122.
5. O’Brien Laramy, Matthew et al. “Chemistry, manufacturing and controls strategies for using novel excipients in lipid nanoparticles.” *Nature nanotechnology*, 2025, Jan 16.
6. Rele, Shyam. “COVID-19 vaccine development during pandemic: gap analysis, opportunities, and impact on future emerging infectious disease development strategies.” *Human vaccines & immunotherapeutics*, 2021, 17,4: 1122-1127.
7. Mehta, Meenu et al. “Lipid-Based Nanoparticles for Drug/Gene Delivery: An Overview of the Production Techniques and Difficulties Encountered in Their Industrial Development.” *ACS materials Au*. 2023, 3,6 600-619.
8. Wang, Chang et al. “Blood-brain-barrier-crossing lipid nanoparticles for mRNA delivery to the central nervous system.” *Nature materials*, 2025, 10.1038/s41563-024-02114-5.
9. Short, Lauralie et al. “Direct in vivo CAR T cell engineering.” *Trends in pharmacological sciences* vol. 45,5 (2024): 406-418. doi:10.1016/j.tips.2024.03.004
10. Rurik, Joel G et al. “CAR T cells produced in vivo to treat cardiac injury.” *Science (New York, N.Y.)* vol. 375,6576 (2022): 91-96. doi:10.1126/science.abm0594
11. Vaidya, Amita, et al. “Analytical characterization of heterogeneities in mRNA-lipid nanoparticles using sucrose density gradient ultracentrifugation”. *Analytical Chemistry*, 2024, 96.14: 5570-5579.
12. Bepperling, Alexander et al. “Determination of mRNA copy number in degradable lipid nanoparticles via density contrast analytical ultracentrifugation.” *European Biophysics Journal*, 2023, 52.4: 393-400.
13. Counil, Hermine et al. “Brain penetration of peripheral extracellular vesicles from Alzheimer’s patients and induction of microglia activation.” *J Extracell Biol*, 2025, 4(1):e70027.

BECKMAN COULTER Life Sciences

For Beckman Coulter’s worldwide office locations and phone numbers, please visit Contact Us at beckman.com
2025-GBL-EN-107507-v1

danaher