



FROM FORMULATION TO MANUFACTURING: LIPID NANOPARTICLE mRNA VACCINES, GENE THERAPIES & OTHER NANOMEDICINES

In this article, Stephen Allan, Health Care Communications at Evonik, reviews the history and formulation advantages of lipid nanoparticles (LNPs) and addresses factors that should be considered by pharmaceutical companies during the CDMO selection process to help reduce regulatory risk, improve product performance and accelerate speed to market.

INTRODUCTION

Lipid nanoparticles (LNPs) have received significant attention in recent months due to their use as the preferred delivery technology for several messenger RNA (mRNA)-based vaccine candidates that are being developed for the prevention of COVID-19. The ability of

lipid nanoparticles to encapsulate genetic material including mRNA, as well as a range of other biologically active agents, for controlled delivery to a target cell or organ site, has now been clinically proven over almost 30 years of commercial use. This long history of clinical performance, together with their ability to be rapidly developed and scaled-up into a finished product, has made LNPs the *de facto* standard for gene- and cell-based therapies and other nanomedicines. In addition to mRNA vaccines, LNP-based formulations have become the gold standard for the development of many complex parenteral products such as anticancer agents, antibiotics, drug combinations and personalised medicines.

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1995 (see Table 1), around half have received the support of Evonik’s team for advanced drug delivery.

The development of gene-based therapies, such as mRNA vaccines and other nanomedicines, requires the use of delivery technologies that are not only safe and efficacious, but simple to customise and efficient to manufacture. Such technologies must also be able to penetrate target cells effectively and ensure release for reliable systemic or local delivery. While delivery systems involving siRNA-conjugates have received some attention for use with a few highly specialised application areas, the overwhelming majority of non-viral formulations developed to date for gene therapies, cell targeting and other nanomedicines have leveraged LNP drug delivery technology.



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Product	Year Approved	API
Doxil®	1995	doxorubicin
Daunoxome®	1996	daunorubicin
AmBisome®	1997	amphotericin B
Visudyne®	2000	verteporfrin
Definity®	2001	octafluoropropane
Myocet®	2002	doxorubicin
DepoCyte®	2002	cytarabine
DepoDur®	2004	morphine
Mepact®	2009	mifamurtide (MTP-PE)
Exparel®	2011	bupivacaine
Marqibo®	2012	vincristine
Onivyde®	2015	irinotecan
Vyxeos®	2017	cytarabine and daunorubicin
Arikyce®	2018	amikacin
Onpattro®	2018	patisiran
Generic Doxil (Sun Pharma, Dr. Reddy's)	2001/2013 (Sun);	doxorubicin

Table 1: Liposome-based products approved since 1995.

Cell membranes are largely composed of lipids, which possess amphipathic qualities, whereby molecules contain one part that is water insoluble and another that is water soluble (Figure 1). While such molecules can be dried as powders or oils, they can be easily rehydrated in water whereby they self-assemble into higher-



Figure 1: A liposome encapsulating an aqueous core and a bilayer consisting of hydrophobic acyl chains.

order spherical vesicles known as liposomes. These naturally occurring cellular processes are emulated in drug delivery systems that are capable of entrapping and retaining therapeutic or diagnostic agents within the liposome (Figure 2).

Lipid particles can be reliably processed via extrusion or micro-mixing systems into

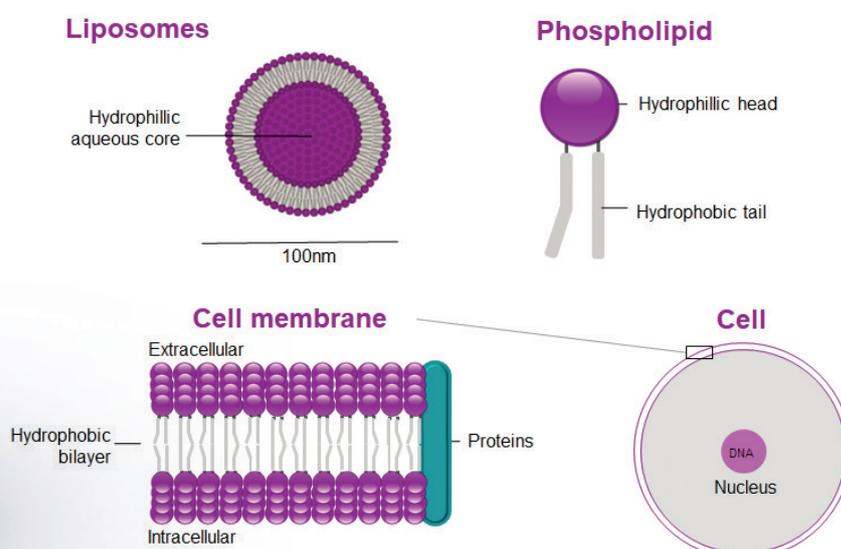


Figure 2: Liposomes mimic naturally occurring and highly stable vesicles featuring a cell-like morphology and high biocompatibility.

vesicle sizes down to, and in some cases even below, 50 nm. Furthermore, they can be precisely tuned to release the product payload at a rate that is therapeutically optimised for the silencing of targeted genes or the expression of therapeutic proteins. LNPs can also be tailored to exhibit specific physicochemical properties, such as particle size and surface charge, to satisfy a variety of functional requirements.

The Clinical & Commercial History of LNPs

Virtually any biologically active agent has the potential to be formulated with LNPs including hydrophobic drugs, small molecules, proteins and peptides, oligonucleotides and mRNA (Figure 3).

This ability for LNPs to encapsulate and protect a payload against degradation, while safely enhancing biodistribution and solubility characteristics, was first applied in highly potent compounds. Examples include intravenous administration of oncology drugs to help increase particle accumulation at the site of a solid tumour. These initial studies led to the approval of the first commercial LNP-based anticancer drug, Doxil, (doxorubicin, J&J) in 1995. Several other small-molecule drugs have been approved subsequently for oncology applications using LNPs to encapsulate and transport active molecules.

Of the almost 20 LNP-based drug formulations that have now been approved for human use (Table 1), most feature relatively simple formulations where the role of the LNP is to provide sufficient protection and stability so that the drug can be retained in the circulatory system and mediate controlled release at the target delivery site.

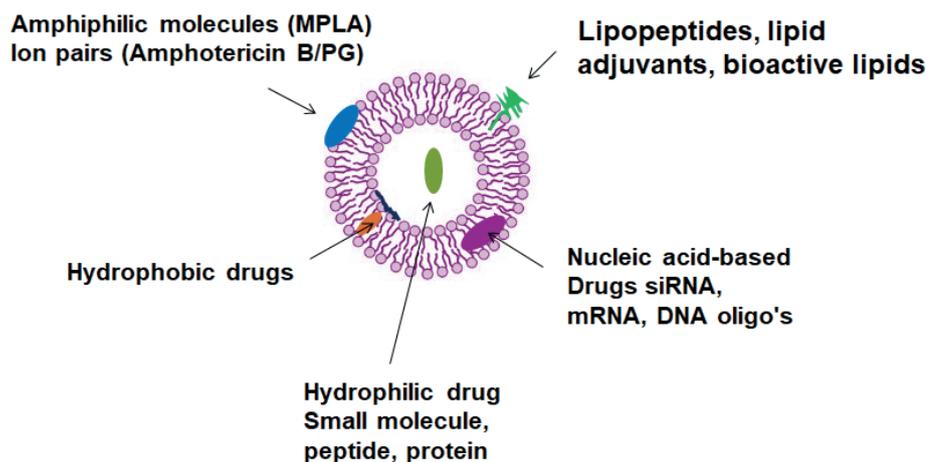


Figure 3: Liposomes provide excellent formulation versatility.

However, the potential of LNPs to deliver specialised or personalised drug products attracted significant industry attention in 2018 following the approval by the US FDA of Alnylam Pharmaceuticals' Onpatro® (patisiran lipid complex injection) for the treatment of polyneuropathy in people with the rare disease hereditary transthyretin-mediated amyloidosis. Onpatro® ushered in a new era of medicines collectively referred to as non-viral gene therapies.

In the case of Onpatro®, LNPs are first used to stabilise and then deliver small nucleic acid fragments known as short interfering RNA (siRNA) into the cytoplasm of the target cells in the liver. The delivered siRNA molecules then promote the degradation of a specific mRNA molecule. By decreasing the amount of mRNA, the ability of the cell to produce that specific protein within the liver is impaired. Such processes represent a new area of therapeutic intervention where faulty or over-expressed proteins can trigger certain genetic diseases, and decreasing their expression has therapeutic benefit.

Developing LNP-Based Formulations for use With RNA Applications

Biological fluids can rapidly degrade "naked" RNA molecules before they reach the target site. LNPs therefore play a vital role in encapsulating and protecting these highly active, but easily degradable, payloads until the target tissue is reached, thus ensuring the silencing of specific genes or the expression of therapeutic proteins. When properly formulated and manufactured, LNP-based formulations for RNA applications can significantly increase the effectiveness of delivery to the target site and the corresponding rate of cellular uptake.

One of the key considerations taken in the development of an LNP-based formulation with specific functional properties is the composition of the lipid components. For example, ionisable cationic lipids are generally responsible for maximising the intracellular delivery of the nucleic acid and play a role in payload encapsulation. The length, level of unsaturation and linker moiety of the hydrocarbon chains and pK values for the lipid are other factors that can influence potency and efficacy.

Another key component in determining LNP functionality is the incorporation of polyethylene glycol-lipids (PEG-lipids), which are comprised of a polymeric PEG chain and two hydrophobic lipid tails. These PEG lipids play a vital role in particle formation and storage stability.

More importantly, they help prevent particle aggregation, modulate interactions with blood proteins and prevent rapid degradation by the immune system.

Manufacturing Methods for LNP-Based Formulations

The typical manufacturing process for liposome formulations is comprised of four steps that can be summarised as: formation, size reduction, purification and sterile filtration. The initial formation of the crude liposome suspension and its size reduction can be achieved most reliably via either a solvent dilution process followed by extrusion or microfluidic (micromixing) processes, which is a single-step process to achieve target nanometre size ranges. Extrusion is a pressure filtration process, where aqueous suspensions of lipids are forced through filters with a defined pore size, optimised for size reduction and high trapping efficiency. LIPEX® extruders have been the industry standard in this regard for more than two decades and range in scale from benchtop to commercial production for liposomes encapsulating various payloads including small molecules, proteins and peptides (Figure 4).

However, while such extrusion-based processes are preferred for many liposomal formulations, their application for use with mRNA and other nucleic acid systems has proven to be challenging. Increasingly, the most popular process for mRNA-LNP synthesis involves rapid mixing whereby a water-miscible organic phase containing

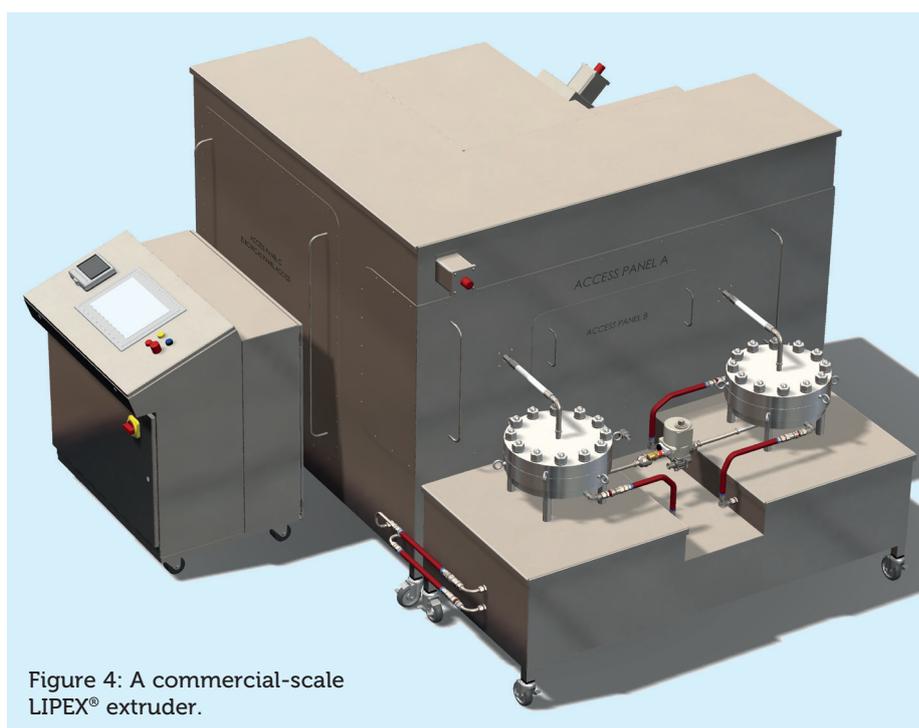


Figure 4: A commercial-scale LIPEX® extruder.

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the lipid components is mixed at dilute concentrations with an acidic aqueous solution containing the nucleic acid. A typical micromixing system consists of a set of pulse-free pumps and a mixing unit such as a T-connector or microfluidic chip. All micro-mixing systems require large in-process volumes and must operate at high flow rates to avoid long process times.

Following size reduction, purification typically occurs with tangential flow filtration systems to remove solvents, as well as any un-entrapped materials and buffer exchange. A clarifying step through a filter is then carried out for bioburden reduction, and also to remove any larger particles (>0.2 µm). As a final step prior to aseptic filling, sterile filtration commonly occurs through 0.2 µm filters to narrow particle distribution further.

Future Applications for LNPs

LNPs are now widely accepted across pharmaceutical and biotechnology industries as an advanced and commercially

proven delivery system that is enabling the commercialisation of gene therapies on an unprecedented scale. Accordingly, LNPs have helped to herald the arrival of a new era of medicine where genetic diseases can be effectively treated or cured, and where vaccines can be produced within the body rather than by the purification of non-infectious viruses. Significant opportunities exist for LNP-based formulations to create drug products for protein replacement therapy, for preventative or curative vaccines and for gene editing purposes.

In addition to RNA, DNA and siRNA-based therapeutics, LNP-based formulations are being increasingly considered for use across a variety of other application areas including anticancer agents and antibiotics, peptide and protein-based synthetic vaccines, ligand-targeted formulations and imaging contrast agents.

Over the coming decade, LNPs are also expected to enable the development of even more complex nanomedicines. Examples include the development of drug combination products, synthetic vaccines or immunotherapies where LNP-based formulations are required to ensure the co-presentation of multiple components into the target cell. Additional opportunities include combining LNPs with tissue sequencing and other functional technologies to help create personalised or custom-made formulations that can enable outcomes such

as the insertion of tumour-associated neo-antigens into mRNA vaccines.

Critical Factors in the Selection of a CDMO Partner for LNP-Based Drug Products

Given the highly specialised nature of developing LNP-based drug products, it is common for pharmaceutical companies to partner with contract development and manufacturing organisations (CDMO) that have established core competencies and a proven record for performance within this technology area. Such strategic partnerships may not only span formulation and process development activities, but also the clinical and commercial manufacturing of the finished product. Accordingly, it is important that CDMOs have significant expertise for the manufacturing of LNP-based formulations.

Prospective CDMO partners should be able to provide the customer with non-confidential demonstrations of how they have been able to help a customer deliver project outcomes within either the same or an equivalent application area. For mRNA or other nucleic acid-based projects, such case studies may include the successful development and production of formulations that ensure a payload is not degraded until arrival at the target site. Other demonstrated project outcomes may include increasing the solubility of lipophilic APIs, reducing systemic toxicity, or enhancing biodistribution as well as cellular and tissue uptake.

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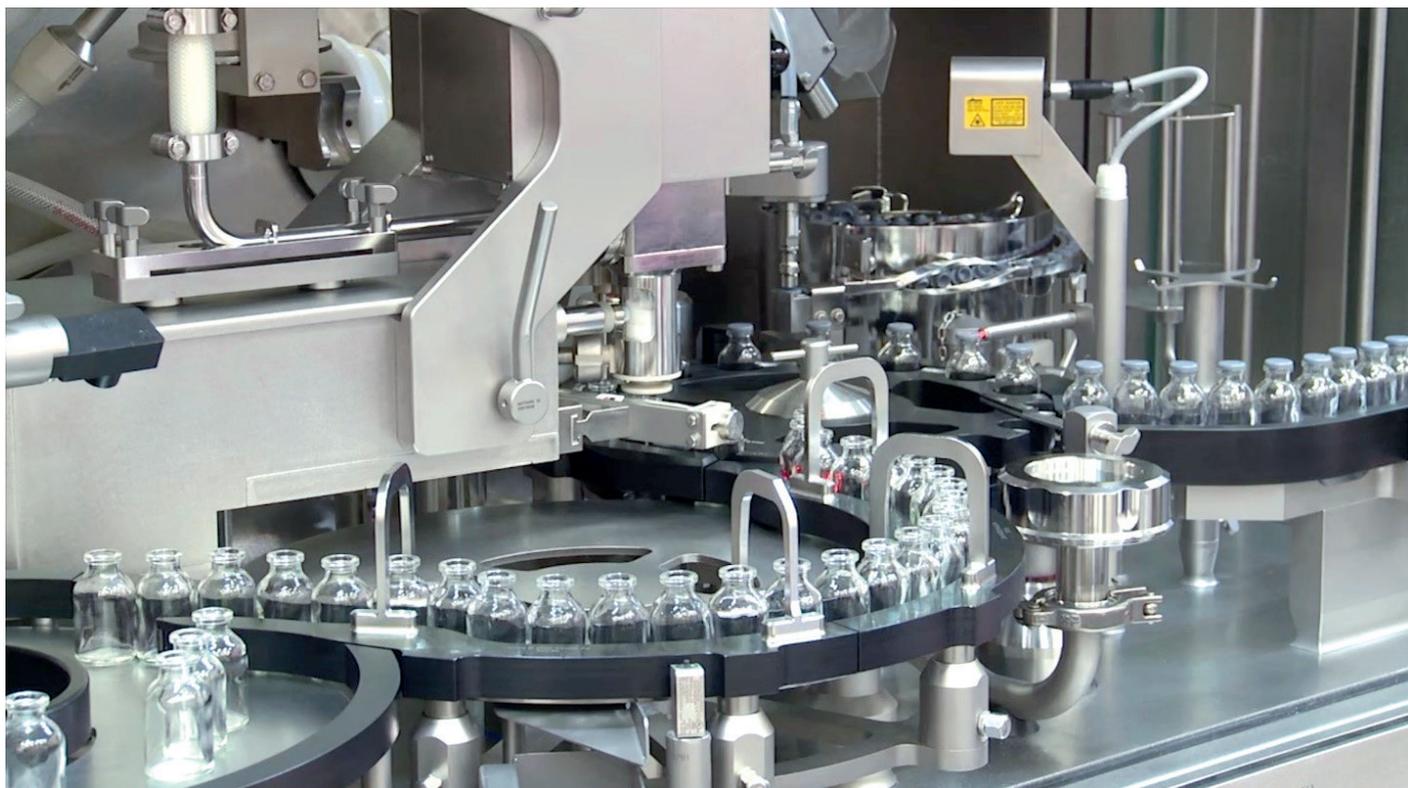


Figure 5: A modular, automated aseptic line at Evonik's Birmingham (AL, US) site for the filling of powders, liquids or suspensions into vials.

Evonik is a global CDMO for advanced drug delivery. For complex parenteral drug products, the company provides a broad portfolio of functional excipients and a range of CDMO and aseptic filling services compatible with more than six drug delivery technology platforms. In addition to LNPs, these platforms include polymeric nanoparticles, nanoparticles, micelles, implants and *in situ* forming. For LNP-based drug products, Evonik has more than 25 years of industry experience through its acquisition and integration of the Canadian CDMO Transferra NanoSciences (previously Northern Lipids). Approximately 50% of all LNP-based drug products approved to-date have received the support of either Evonik or Transferra.

Evonik has developed hundreds of LNP-based formulations for gene and cell-based therapies as well as other nanomedicines with a broad, global base of pharmaceutical and biotech companies. A range of CDMO services are available to support customer projects from drug discovery and preclinical studies through to the large-scale GMP production and aseptic filling of the final drug product. The company provides its LIPEX® extruders ranging from benchtop to commercial production scale, and also has extensive in-house manufacturing capabilities as well as other industry relationships for micromixing (Figure 5).

Since the acquisition of Transferra, Evonik has transformed its Vancouver (Canada) facility into a centre of excellence for drug products that require liposomal and nanoparticle technologies for delivery. These investments have effectively doubled the size of the company's Vancouver site, and considerably expanded manufacturing and formulation development capabilities that are available to support new customer projects. In parallel, Evonik has begun to harmonise the equipment and processes at both its Vancouver site and its late stage/commercial manufacturing site for complex parenteral drug products in Birmingham, AL, US. Through these and other investments, Evonik is now positioned to assist pharmaceutical customers in the development of liposomal-based parenteral drug products to support their entry into human clinical trials and their scale-up for commercial use.

ABOUT THE COMPANY

Evonik is one of the world's leading CDMOs for advanced drug delivery. For oral and parenteral drug products, Evonik provides customers with a broad portfolio of functional excipients, drug delivery technologies, formulation development, process development and cGMP manufacturing services. As a CDMO for complex parenterals, Evonik has more than three decades of expertise with drug delivery technologies including polymeric microparticles and nanoparticles, lipid nanoparticles, drug-loaded implants, micelles and *in situ* forming. Evonik is also a leader for process technologies including micro-encapsulation, precise hot-melt extrusion and liposomal extruders. Additional services include the contract manufacturing of APIs and intermediates, and the supply of amino acids plus other pharmaceutical and cell culture ingredients.

ABOUT THE AUTHOR

Stephen Allan is a healthcare communication specialist with more than 20 years of expertise across global drug delivery, medical device and nutraceutical markets. After graduating in journalism, he has co-ordinated communication activities for Australian, US and European-based companies. Based in Germany since 2017, Mr Allan supports global communication activities for the Evonik Health Care business line.