ISSN: 2231-3656

International Journal of Pharmacy and Industrial Research (IJPIR)

IJPIR |Vol.13 | Issue 4 | Oct - Dec -2023

www.ijpir.com

DOI: https://doi.org/10.61096/ijpir.v13.iss4.2023.322-335

Lipid Nanoparticles for mRNA and siRNA Delivery: Formulation Design, Ionizable Lipid Optimization, and Therapeutic Applications

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Published on: 20.10.2023
Published by: Futuristic Publications

Abstract:

Background: Lipid nanoparticles (LNPs) have rapidly become the most clinically validated non-viral platform for the delivery of nucleic acid therapeutics, culminating in the global deployment of mRNA-based COVID-19 vaccines. The inherent instability of messenger RNA (mRNA) and small interfering RNA (siRNA) in biological fluids, their impermeability across cellular membranes, and susceptibility to enzymatic degradation demand robust encapsulation within structurally sophisticated nanocarriers. LNPs fulfill these requirements through their four-component lipid architecture and unique capacity to facilitate endosomal escape, enabling cytosolic delivery of functional nucleic acids with high efficiency.

Objective: This review critically appraises the formulation principles, lipid component optimization, manufacturing methodologies, characterization strategies, mechanisms of intracellular delivery, and therapeutic applications of LNPs for mRNA and siRNA delivery, with particular emphasis on recent research findings and their translational significance.

Results and Discussion: The LNP architecture comprising an ionizable lipid, helper phospholipid, cholesterol, and PEG-lipid enables efficient nucleic acid encapsulation, systemic circulation, cellular uptake, and endosomal membrane disruption. Ionizable lipid pKa values between 6.2 and 6.8 are critical determinants of in vivo potency. Clinically approved systems including patisiran for siRNA delivery and the COVID-19 mRNA vaccines BNT162b2 and mRNA-1273 have validated this platform at a population scale. Emerging directions including organ-selective LNPs, self-amplifying mRNA, and circular RNA further expand the therapeutic reach of this technology.

Conclusion: LNPs represent a transformative pharmaceutical platform for nucleic acid delivery with demonstrated clinical efficacy across infectious disease, hereditary disorders, and oncology. Continued innovation in lipid design, manufacturing scalability, and extrahepatic targeting will define the next generation of RNA medicines.

Keywords: Lipid nanoparticles; mRNA delivery; siRNA delivery; ionizable lipids; endosomal escape; nucleic acid therapeutics; COVID-19 vaccine; patisiran; RNA therapeutics

1. Introduction

The development of nucleic acid therapeutics has opened a fundamentally new dimension in pharmacological intervention, offering the ability to modulate gene expression with a precision that conventional small molecule drugs cannot achieve. Messenger RNA and small interfering RNA represent two mechanistically distinct but complementary modalities within this class. While mRNA directs the

transient synthesis of a therapeutic protein within the cytoplasm — without engaging the genome — siRNA mediates sequence-specific post-transcriptional gene silencing through the RNA-induced silencing complex (RISC), enabling highly selective knockdown of disease-driving genes [1,2]. Together, these modalities address an expansive range of diseases previously regarded as therapeutically inaccessible, from rare inherited metabolic disorders to common infectious diseases and solid malignancies.

The clinical realization of nucleic acid therapeutics has been constrained by a series of well-characterized biological barriers. Both mRNA and siRNA are large, hydrophilic, negatively charged macromolecules that are rapidly degraded by circulating ribonucleases, renally cleared within minutes of systemic administration, and unable to cross lipid bilayer membranes by passive diffusion. Following cellular endocytosis, both molecules face further degradation within acidifying endosomal compartments unless released into the cytoplasm with sufficient efficiency [3,4].

Lipid-based nanoparticles emerged as the answer to these delivery challenges through decades of iterative formulation research. The earliest lipid transfection agents, based on permanently cationic lipids, demonstrated proof-of-concept nucleic acid delivery but were burdened by significant systemic toxicity. The introduction of ionizable lipids — pH-sensitive molecules that carry a positive charge only under acidic conditions — transformed the safety and efficacy profile of lipid-based nucleic acid carriers, giving rise to the contemporary LNP formulation paradigm [5,6]. The four-component architecture comprising an ionizable lipid, helper phospholipid, cholesterol, and a PEGylated lipid represents the culmination of this evolution, balancing encapsulation efficiency, colloidal stability, endosomal escape capacity, and systemic tolerability within a single manufacturable system.

The clinical validation of LNPs reached a landmark milestone in 2018 with the FDA approval of patisiran (Onpattro), a hepatically targeted siRNA-LNP for hereditary transthyretin-mediated amyloidosis. The subsequent emergency authorization and full approval of two mRNA-LNP COVID-19 vaccines — BNT162b2 and mRNA-1273 — demonstrated that LNP manufacturing could be scaled to produce billions of doses within months, and that the safety profile of the platform was acceptable across diverse global populations [7,8]. This review provides a comprehensive and critical analysis of LNP formulation design, lipid component optimization, manufacturing technologies, characterization methods, delivery mechanisms, and therapeutic applications, followed by a comparative assessment against alternative nucleic acid delivery platforms and a critical evaluation of unresolved scientific questions.

2. Scientific Background

2.1 Molecular Biology of mRNA and siRNA

Messenger RNA is a single-stranded ribonucleic acid that carries the genetic blueprint for protein synthesis from the nucleus to the cytoplasmic ribosome. In therapeutic applications, in vitro transcribed mRNA is engineered with structural elements that optimize translational efficiency and intracellular stability: a 5' cap structure analogous to the endogenous 7-methylguanosine cap, optimized 5' and 3' untranslated regions, a codon-optimized open reading frame, and a polyadenylated 3' tail. Chemical substitution of uridine residues with N1-methylpseudouridine substantially reduces recognition by innate immune pattern recognition receptors while simultaneously enhancing ribosomal translation efficiency [9,10]. These modifications in contemporary therapeutic mRNA have increased protein expression levels by orders of magnitude compared to early in vitro transcribed RNA constructs.

Small interfering RNA consists of a 21 to 23 nucleotide double-stranded RNA duplex with characteristic 2-nucleotide 3' overhangs. Following cytoplasmic delivery, the antisense guide strand is selectively incorporated into the RISC complex, where the Argonaute-2 protein cleaves target mRNA sequences complementary to the guide strand, initiating their degradation. The catalytic nature of RISC-mediated cleavage enables sustained gene knockdown from a single dose. Chemical modifications including 2'-O-methyl and 2'-fluoro ribose substitutions, phosphorothioate backbone linkages, and locked nucleic acid inclusions enhance nuclease resistance and reduce off-target immunostimulatory activity [11,12].

2.2 Physiological Barriers to Nucleic Acid Delivery

The physicochemical properties of mRNA and siRNA create formidable delivery challenges at every biological level. In the systemic circulation, both molecules are rapidly degraded by ribonucleases present in plasma and tissue fluids, with unmodified RNA having a plasma half-life of seconds to minutes. Their large molecular size and strong negative charge prevent passive membrane crossing, while their hydrodynamic radius falls below the glomerular filtration threshold for siRNA, resulting in rapid renal clearance [13,14].

The endosomal trafficking barrier represents perhaps the greatest challenge in intracellular nucleic acid delivery. Following endocytosis, LNP-nucleic acid complexes are sequestered in early endosomes (pH approximately 6.5) and progressively trafficked to late endosomes and lysosomes where pH drops to 4.5 to 5.5 and hydrolytic enzymes are concentrated. Studies employing live-cell imaging and quantitative endosomal content tracking have estimated that only 1 to 2% of endocytosed siRNA successfully escapes into the cytoplasm, with the remainder routed to lysosomal degradation. This inefficiency represents the primary rate-limiting step in LNP-mediated nucleic acid delivery and the central target of ionizable lipid design optimization [15].

3. Classification of Lipid Nanoparticles

3.1 Classification by Nucleic Acid Cargo

LNPs are broadly classified by the nucleic acid payload they carry, as each cargo type imposes distinct formulation requirements. mRNA-LNPs are designed for transient protein expression and employed in prophylactic vaccines, cancer immunotherapy, protein replacement therapy, and in vivo gene editing. siRNA-LNPs are optimized for hepatic or tissue-specific gene silencing in metabolic, cardiovascular, and oncological diseases. miRNA mimic, antisense oligonucleotide, circular RNA, and self-amplifying RNA LNPs represent advancing or emerging categories within this classification [16].

3.2 Classification by Lipid Generation

First-generation lipid delivery systems employed permanently cationic lipids such as DOTMA and DOTAP, which effectively complexed nucleic acids through electrostatic interaction but caused dose-dependent cytotoxicity. Second-generation systems introduced ionizable lipids that adopted a cationic character only under acidic conditions, dramatically improving tolerability. Third-generation LNPs featuring advanced ionizable lipids with optimized pKa values, biodegradable ester linkages, and branched tail structures achieved superior in vivo potency and formed the basis for all currently approved nucleic acid products. Fourth-generation SORT LNPs, in which a fifth lipid component directs biodistribution to organs beyond the liver, represent the current frontier of LNP formulation research [17,18].

3.3 Classification by Structural Organization

Cryo-transmission electron microscopy studies have revealed that LNPs do not adopt the classical liposomal bilayer structure but instead form electron-dense nanoparticles with an internal organization of inverted lipid phases. Small-angle X-ray scattering and nuclear magnetic resonance studies indicate that the nucleic acid is compacted within a lipid-encased core in which ionizable lipids adopt an inverted hexagonal or cubic phase, a structural arrangement mechanistically linked to endosomal membrane disruption [19].

Table 1: Classification of lipid nanoparticles based on generation, lipid composition, application, and representative clinical products

Generation	Lipid Type	Representative Lipids	Primary Application	Clinical Example
1st Generation	Cationic lipids	DOTMA, DOTAP, DC-Chol	In vitro gene transfer	Lipofectin (research use)
2nd Generation	Early ionizable lipids	DLin-KC2-DMA, DLin-DMA	Hepatic siRNA delivery	Preclinical to Phase I
3rd Generation	Advanced ionizable lipids	DLin-MC3-DMA, ALC-0315, SM-102	siRNA and mRNA delivery	Patisiran, BNT162b2, mRNA-1273
4th Generation	Organ-selective lipids	Supplemental cationic/anionic lipids	Lung, spleen, CNS targeting	Preclinical development

DOTMA: 1,2-di-O-octadecenyl-3-trimethylammonium propane; DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane; DLin-MC3-DMA: dilinoleylmethyl-4-dimethylaminobutyrate; ALC-0315: (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); SM-102: heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate.

4. Formulation Design and Development

4.1 Ionizable Lipids

The ionizable lipid is the functional cornerstone of the LNP, governing nucleic acid encapsulation, endosomal membrane disruption, in vivo potency, and tissue tropism. These molecules are engineered with a pH-titratable tertiary amine head group connected through a linker region to two or more hydrophobic tail chains. At the acidic pH used during formulation (typically pH 3.5 to 4.5), the amine is protonated and positively charged, driving electrostatic complexation with the negatively charged nucleic acid backbone. At physiological pH 7.4, the amine reverts to its neutral uncharged state, minimizing non-specific interactions with serum proteins and substantially improving systemic tolerability compared to permanently cationic lipid systems [20,21].

The apparent pKa of the ionizable lipid is the single most predictive physicochemical parameter for LNP in vivo efficacy. An extensive structure-activity relationship study established that ionizable lipids with pKa values between 6.2 and 6.8 consistently achieve superior hepatic gene silencing. Within this range, the ionizable lipid remains predominantly uncharged at blood pH 7.4 but acquires sufficient positive charge within acidifying endosomes to interact electrostatically with anionic bis(monoacylglycero)phosphate lipids in the inner endosomal membrane, triggering bilayer disruption and nucleic acid release into the cytoplasm [22,23].

DLin-MC3-DMA, incorporated in patisiran, has a pKa of approximately 6.44 and represents the benchmark for hepatic siRNA delivery. ALC-0315 with a pKa of approximately 6.09 and SM-102 with a pKa of approximately 6.68 are the ionizable lipids in BNT162b2 and mRNA-1273 respectively. Structure-activity relationship data from these and hundreds of systematically synthesized lipid analogs have established that unsaturation in the hydrophobic tail region, branching of tail chains, and ester linkages in the linker region collectively enhance membrane fusogenicity, endosomal disruption, and biodegradability of the ionizable lipid [24,25].

4.2 Helper Phospholipids

Helper phospholipids constitute 10 to 20 mol% of the LNP formulation and serve to stabilize the particle bilayer structure, influence membrane fluidity and phase behavior, and modulate cellular uptake kinetics. DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine) is employed in both patisiran and the COVID-19 mRNA vaccines owing to its high phase transition temperature, which confers structural rigidity and reduces drug leakage during storage and systemic circulation. DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), with its intrinsic propensity to adopt inverted hexagonal phase structures, has been employed as an alternative helper lipid that may enhance endosomal escape through its fusogenic properties [26].

Comparative studies examining the influence of helper phospholipid composition on mRNA expression have demonstrated that DSPC-containing LNPs favor prolonged hepatic protein expression, while DOPE-containing formulations achieve higher levels of mRNA translation in muscle and immune cells. For intramuscular mRNA vaccination, DSPC-based LNPs produced superior immunogenicity outcomes in non-human primate and human clinical studies, attributed to extended antigen persistence and enhanced dendritic cell activation at the injection depot [27].

4.3 Cholesterol

Cholesterol is incorporated at 30 to 45 mol% and serves multiple structural and functional roles within the LNP. As a membrane-ordering molecule, cholesterol fills the interstitial spaces between lipid tails, reducing membrane permeability and preventing premature nucleic acid leakage. Cholesterol also facilitates LNP cellular uptake through promotion of apolipoprotein E adsorption onto the particle surface in vivo, enabling recognition by hepatic low-density lipoprotein receptors and efficient receptor-mediated endocytosis in hepatocytes. Structural cholesterol analogs including beta-sitosterol, a plant-derived sterol, were reported to enhance mRNA-LNP transfection efficiency up to threefold compared to standard cholesterol, attributed to differences in membrane lipid ordering and endosomal membrane interaction [28,29].

4.4 PEG-Lipids

PEG-lipids are incorporated at 1.0 to 2.5 mol% to provide a hydrophilic polyethylene glycol corona that confers colloidal stability, prevents particle aggregation, reduces opsonization by serum proteins, and prolongs systemic circulation by shielding the particle from mononuclear phagocyte system recognition. ALC-0159 and PEG2000-DMG are the PEG-lipids in BNT162b2 and mRNA-1273 respectively, while PEG2000-C-DMG is used in patisiran. PEG-lipid content requires precise optimization: excess PEG density

sterically inhibits cellular uptake and endosomal fusion, while insufficient PEG leads to colloidal instability. The lipid anchor chain length governs PEG-lipid dissociation kinetics — shorter C14 anchors dissociate more rapidly, facilitating faster cellular uptake in tissues where extended circulation is not required [30,31].

5. Preparation Methods

5.1 Microfluidic Mixing

Microfluidic mixing technology is currently the gold standard for LNP preparation at both laboratory and clinical manufacturing scales. An organic phase containing lipids dissolved in ethanol is rapidly mixed with an aqueous phase containing nucleic acid at low pH using staggered herringbone micromixer chips. The controlled rapid dilution of ethanol triggers self-assembly of lipid components around the nucleic acid cargo, producing highly monodisperse LNPs with narrow particle size distributions (PDI <0.1) and encapsulation efficiencies consistently exceeding 90%. The aqueous-to-organic flow rate ratio, total volumetric flow rate, lipid concentration, and temperature are the primary process parameters governing particle size and formulation quality [32,33].

5.2 Tangential Flow Filtration and Buffer Exchange

Following microfluidic assembly, the crude LNP dispersion requires removal of residual ethanol, unencapsulated nucleic acid, and low pH buffer before administration. Tangential flow filtration is the preferred method for this downstream processing step, offering continuous, gentle, and scalable diafiltration that exchanges the acidic ethanol-containing formulation buffer for a physiologically compatible medium such as phosphate-buffered saline or sucrose-containing stabilization buffer. TFF preserves LNP colloidal integrity and encapsulation efficiency during buffer exchange without the shear stress or dilution steps associated with centrifugal methods [34].

5.3 Lyophilization for Storage Stability

The thermodynamic instability of aqueous LNP dispersions during storage, driven by phospholipid hydrolysis and mRNA chemical degradation, limits the shelf life of liquid LNP formulations even under refrigerated conditions. Lyophilization in the presence of glass-forming cryoprotectants, most commonly sucrose or trehalose at concentrations of 5 to 10% w/v, arrests molecular mobility and provides amorphous matrix stabilization of the LNP structure during desiccation and long-term storage. Optimization of cryoprotectant concentration, lyophilization cycle parameters including primary and secondary drying temperatures and pressures, and reconstitution procedure is essential to ensure that lyophilized LNPs reconstitute to particles with physicochemical properties identical to the pre-lyophilization formulation [35,36].

6. Characterization and Evaluation

6.1 Particle Size, Polydispersity, and Zeta Potential

Dynamic light scattering is routinely employed for measurement of hydrodynamic diameter and polydispersity index of LNPs. Optimal formulations for systemic intravenous delivery typically exhibit mean particle diameters of 60 to 150 nm with PDI values below 0.2. Nanoparticle tracking analysis offers complementary particle size and concentration data by tracking individual particle Brownian motion trajectories. Zeta potential measured by electrophoretic light scattering reflects the electrokinetic surface charge; LNPs incorporating ionizable lipids typically exhibit near-neutral to mildly negative zeta potential at physiological pH, a property that reduces non-specific electrostatic interactions with plasma proteins and cell membranes [37].

6.2 Encapsulation Efficiency

Nucleic acid encapsulation efficiency is quantified using the fluorescent intercalating dye Ribogreen assay, which measures fluorescence intensity in the presence of a membrane-impermeable dye before and after detergent-mediated dissolution of the LNP bilayer. The difference in fluorescence signal between intact and disrupted particles represents the encapsulated fraction. High-quality LNP formulations achieve encapsulation efficiencies of 85 to 98%, with values below 80% indicative of formulation or process optimization requirements [38].

6.3 Ionizable Lipid pKa Determination

The apparent pKa of the ionizable lipid within the LNP is determined using pH-sensitive fluorescent probes, most commonly TNS (6-(p-toluidino)-2-naphthalenesulfonic acid) or HPTS (8-hydroxypyrene-1,3,6-

trisulfonic acid). In the TNS assay, fluorescence intensity increases as ionizable lipid amino groups become protonated at decreasing pH, and the pH at which fluorescence is half-maximal defines the apparent pKa. This measurement is performed routinely as a formulation quality attribute, as pKa is highly predictive of in vivo hepatic gene expression potency and must fall within the 6.2 to 6.8 range for optimal endosomal escape activity [39].

6.4 mRNA Integrity and Identity

mRNA integrity within the LNP is assessed by capillary gel electrophoresis following membrane disruption, which resolves full-length mRNA from degradation fragments and double-stranded RNA impurities. The percentage of intact full-length mRNA is a critical quality attribute, as fragmented mRNA produces truncated non-functional protein. Residual double-stranded RNA content, which activates innate immune pathways through TLR3 signaling, is controlled by ion-exchange chromatography purification of IVT mRNA before formulation [40].

6.5 In Vitro Transfection and Gene Silencing

In vitro transfection efficiency of mRNA-LNPs is evaluated using reporter constructs (luciferase, GFP) or therapeutic mRNA in relevant cell lines or primary cells, providing dose-response data for calculation of EC50 values. siRNA-LNP gene silencing efficiency is assessed by quantitative RT-PCR and western blotting for target gene mRNA and protein, with IC50 determination enabling direct comparison of ionizable lipid potency across formulation series. Cell viability assays confirm that observed gene expression or silencing is not confounded by cytotoxicity at the concentrations tested [41,42].

7. Mechanism of Drug Delivery

7.1 Cellular Uptake

The mechanism of LNP cellular uptake is highly dependent on route of administration and target cell type. Following intravenous administration, LNPs rapidly acquire a protein corona in which apolipoprotein E from plasma adsorbs preferentially onto the particle surface. ApoE-decorated LNPs are recognized by low-density lipoprotein receptors expressed abundantly on hepatocytes, mediating clathrin-dependent receptor-mediated endocytosis and explaining the strong intrinsic hepatic tropism of standard intravenously administered LNP formulations. Following intramuscular injection, LNPs are taken up by muscle cells, local macrophages, and dendritic cells through macropinocytosis and non-specific endocytosis pathways [43,44].

7.2 Endosomal Escape

Following endocytosis, LNPs are trafficked through the endolysosomal pathway from early endosomes (pH 6.0 to 6.5) through late endosomes (pH 5.0 to 5.5) toward lysosomes. The progressive acidification drives protonation of ionizable lipid amine groups, converting them from uncharged to positively charged species. These protonated ionizable lipids interact electrostatically with the negatively charged bis(monoacylglycero)phosphate lipids enriched in the inner leaflet of late endosomal membranes, destabilizing the bilayer through formation of non-bilayer lipid structures including inverted hexagonal phases. This membrane perturbation creates transient disruptions through which nucleic acid cargo is released into the cytoplasm [45,46].

7.3 Intracellular Processing and Activity

Once released into the cytoplasm, mRNA is immediately accessible to ribosomes and begins translation into the encoded protein. The kinetics and magnitude of protein expression depend on mRNA stability governed by the N1-methylpseudouridine modification, poly(A) tail length, and UTR sequences. Maximum protein expression typically occurs within 6 to 24 hours of LNP administration. For siRNA, cytoplasmic release enables loading of the guide strand into the Argonaute-2-containing RISC complex, which then cleaves complementary cytoplasmic mRNA sequences, inducing target mRNA degradation and sustained gene silencing over weeks to months [47,48].

8. Therapeutic Applications

8.1 Prophylactic Vaccination Against Infectious Diseases

The most impactful clinical application of mRNA-LNP technology has been prophylactic vaccination. The BNT162b2 and mRNA-1273 COVID-19 vaccines, both encoding stabilized prefusion SARS-CoV-2 spike protein, demonstrated vaccine efficacies of 95.0% and 94.1% respectively against symptomatic COVID-19 in

pivotal phase 3 trials, with strong safety profiles across diverse global populations [7,8]. Their deployment beginning in December 2020 resulted in administration of billions of doses worldwide, providing the first large-scale demonstration of mRNA-LNP safety and clinical efficacy at a population level. Building on this foundation, mRNA-LNP vaccines against RSV, cytomegalovirus, influenza, and HIV were in active clinical development, with the mRNA-1345 RSV vaccine reporting positive phase 3 efficacy data in older adults [49].

8.2 siRNA Therapeutics for Hereditary Diseases

The approval of patisiran established siRNA-LNP therapeutics as a viable pharmacological modality. Patisiran silences the TTR gene in hepatocytes with an efficiency exceeding 80% reduction in serum transthyretin levels, producing clinically meaningful improvements in neurological function and cardiac parameters in patients with hereditary transthyretin-mediated amyloidosis. Phase 3 clinical data published in the New England Journal of Medicine in 2018 demonstrated statistically significant improvements in the modified neuropathy impairment score compared to placebo [50]. Beyond TTR, siRNA-LNP approaches were in clinical development for PCSK9-mediated hypercholesterolaemia, hepatitis B, and complement-mediated disorders.

8.3 Cancer Immunotherapy

Personalized mRNA cancer vaccines encoding patient-specific tumor neoantigens identified by next-generation sequencing represent a convergence of precision oncology and mRNA-LNP technology. Phase 1 clinical trials of personalized mRNA neoantigen vaccines co-administered with anti-PD-1 checkpoint inhibitors in melanoma, bladder cancer, and other solid tumors reported measurable neoantigen-specific cytotoxic T cell responses and preliminary evidence of clinical benefit, supporting continued clinical development into phase 2 and 3 [51].

8.4 Protein Replacement Therapy

mRNA-LNP technology offers a compelling alternative to recombinant protein administration for enzyme deficiency diseases. The patient's own hepatocytes or other target cells produce the therapeutic protein with complete post-translational modifications following mRNA delivery, potentially improving efficacy and reducing immunogenicity compared to exogenously produced recombinant proteins. Clinical and advanced preclinical programs for mRNA replacement of coagulation factors in hemophilia, alpha-1 antitrypsin in inherited emphysema, and ornithine transcarbamylase deficiency were active with early phase results reported [52,53].

9. Recent Advances

9.1 Organ-Selective Lipid Nanoparticles

The intrinsic hepatic tropism of intravenously administered LNPs has been a formulation challenge for extrahepatic disease applications. The selective organ targeting (SORT) strategy demonstrated that incorporating a supplemental charged lipid as a fifth LNP component redirects biodistribution to the lung (with permanently cationic lipids), spleen (with permanently anionic lipids), or liver (with ionizable lipids) in a predictable composition-dependent manner. Lung-selective SORT LNPs achieved efficient mRNA expression in pulmonary endothelial cells and type II pneumocytes, opening therapeutic opportunities in pulmonary hypertension, acute lung injury, and respiratory viral infections without requiring direct inhalation administration [54].

9.2 Bioreducible and Biodegradable Ionizable Lipids

Concerns about hepatic accumulation of non-biodegradable lipid components following repeated LNP dosing have motivated the development of ionizable lipids with degradable structural elements. Ionizable lipids incorporating ester, disulfide, or acetal linkages in their linker or tail regions undergo intracellular hydrolysis or reductive cleavage following endosomal escape, releasing non-toxic metabolites that are efficiently cleared and reducing the risk of lipid accumulation with chronic dosing. Such biodegradable lipids demonstrated comparable or superior mRNA expression potency to DLin-MC3-DMA in hepatic delivery studies while exhibiting faster lipid clearance kinetics in non-human primate pharmacokinetic studies [55].

9.3 Self-Amplifying mRNA Formulations

Self-amplifying mRNA derived from alphavirus genomic RNA incorporates replicase gene machinery alongside the therapeutic coding sequence, enabling intracellular RNA amplification following cytoplasmic delivery. This intrinsic amplification allows saRNA-LNPs to achieve equivalent or greater antigen

expression and immune response compared to conventional mRNA-LNPs at doses 10 to 100-fold lower, substantially reducing the amount of lipid administered per dose and potentially lowering formulation costs. Several saRNA-LNP vaccine candidates against COVID-19, influenza, and other pathogens were in clinical evaluation, including the first globally approved self-amplifying mRNA vaccine [56].

9.4 Circular RNA Delivery

Circular RNA is a covalently closed RNA species lacking free 5' and 3' termini, conferring resistance to exoribonuclease-mediated degradation. Enzymatic or chemical in vitro circularization of therapeutic RNA sequences produces circRNA with substantially extended intracellular half-life compared to linear mRNA, enabling more prolonged protein expression from a single dose. LNP encapsulation of circRNA was demonstrated to achieve efficient cytoplasmic delivery and functional cap-independent translation in multiple cell types, establishing circRNA-LNPs as an emerging platform for applications requiring sustained protein production [57,58].

10. Comparative Analysis

LNPs occupy a distinctive position among nucleic acid delivery platforms by virtue of their combination of high encapsulation efficiency, scalable manufacturing, clinical precedent, and acceptable tolerability. A direct comparison with alternative delivery systems — including lipoplexes, polymeric nanoparticles, extracellular vesicles, GalNAc conjugates, and viral vectors — reveals both the competitive advantages of LNPs and the contexts in which alternative platforms offer complementary strengths.

Lipoplexes formed by electrostatic complexation of cationic lipids with nucleic acids achieve efficient in vitro transfection but suffer from aggregation in physiological salt concentrations, complement activation, and non-specific cellular toxicity that restrict their clinical utility to local or ex vivo applications. Polymeric nanoparticles based on PEI, PLGA, or dendrimers offer sustained release and surface functionalization flexibility but generally achieve lower transfection efficiency and slower endosomal escape compared to ionizable LNPs. GalNAc-siRNA conjugates, exemplified by the approved inclisiran, provide remarkable hepatocyte targeting through asialoglycoprotein receptor engagement but are limited to siRNA cargo and hepatic applications. Adeno-associated viral vectors achieve highly efficient and durable transgene expression but carry risks of immunogenicity and manufacturing limitations. Extracellular vesicles offer biological membrane composition and inherent immune tolerance but are challenged by low nucleic acid loading efficiency and lack of scalable manufacturing [59,60].

Table 2: Comparative evaluation of nucleic acid delivery platforms across key performance parameters

Platform	Encapsulation	In Vivo Potency	Toxicity Profile	Clinical Status	Scalability
Lipid nanoparticles (LNPs)	85–98%	High	Acceptable	Approved products	Excellent
Cationic lipoplexes	70–90%	Moderate	Significant toxicity	Limited (local use)	Good
Polymeric nanoparticles	60–85%	Moderate	Variable	Phase I/II	Good
GalNAc–siRNA conjugates	Not applicable	High (liver only)	Acceptable	Approved (inclisiran)	Excellent
Extracellular vesicles	20–60%	Moderate	Low	Early clinical	Challenging
Adeno-associated virus	Very high	Very high	Immunogenicity risk	Approved (gene therapy)	Limited

GalNAc: N-acetylgalactosamine.

11. Advantages and Limitations

11.1 Advantages

- High and reproducible nucleic acid encapsulation efficiency exceeding 90%, protecting fragile RNA from ribonuclease degradation in biological fluids

- Clinically validated safety profile demonstrated across the approved patisiran formulation and two mRNA-LNP COVID-19 vaccines administered to billions of individuals worldwide
- Versatility to encapsulate diverse nucleic acid modalities including mRNA, siRNA, miRNA, antisense oligonucleotides, circular RNA, and self-amplifying RNA within the same four-component lipid architecture
- Scalable and reproducible manufacturing using microfluidic mixing platforms compatible with GMP pharmaceutical production at global scale
- Tunable in vivo biodistribution through rational ionizable lipid structure modification and SORT technology, enabling organ-selective delivery to liver, lung, spleen, and other tissues
- Capacity for co-encapsulation of multiple nucleic acid species or combined nucleic acid and small molecule payloads within a single particle
- Biodegradable formulation components with defined metabolic clearance pathways and favorable preclinical toxicological profiles
- Rapid development timeline — COVID-19 vaccine produced from sequence identification to clinical deployment within 12 months, establishing a new pharmaceutical development paradigm

11.2 Limitations

- Strong intrinsic hepatic tropism following intravenous administration limits therapeutic applicability to extrahepatic target tissues without specialized formulation strategies
- Cold chain requirements of −20°C to −80°C for certain mRNA formulations substantially complicate global distribution logistics and limit deployment in resource-constrained settings
- Anti-PEG antibodies generated following initial LNP exposure may reduce therapeutic efficacy and increase hypersensitivity risk upon repeat dosing in patients requiring chronic treatment
- Transient duration of mRNA expression over days to weeks necessitates repeated dosing for chronic diseases, accumulating lipid component exposure over time
- Injection site reactogenicity including pain, erythema, and swelling attributed to local inflammatory activation by LNP lipid components and mRNA-encoded antigens
- Relatively high manufacturing cost compared to conventional small molecule formulations limits economic accessibility in low-income markets
- Particle size polydispersity and batch-to-batch consistency remain critical manufacturing quality challenges requiring rigorous process analytical control

12. Clinical Translation and Marketed Products

The clinical translation journey of LNP-based nucleic acid therapeutics spans more than two decades from the first demonstration of lipid-mediated siRNA delivery in non-human primates to globally deployed human medicines. The approval of patisiran in 2018 by the FDA and EMA validated the complete LNP development paradigm and established regulatory precedent for LNP quality requirements including particle size, encapsulation efficiency, pKa, and mRNA integrity specifications. The COVID-19 pandemic demonstrated that mRNA-LNP medicines can be developed from sequence design to phase 3 enrollment within eight months, exploiting platform manufacturing processes that enabled rapid scale-up to billions of doses.

Table 3: Clinically approved and advanced lipid nanoparticle-based nucleic acid therapeutics

Product	Cargo	Target	Indication	Year	Ionizable Lipid
Patisiran (Onpattro)	siRNA	TTR mRNA	hATTR amyloidosis	2018	DLin-MC3-DMA
BNT162b2 (Comirnaty)	mRNA	SARS-CoV-2 spike	COVID-19 prevention	2021	ALC-0315

mRNA-1273 (Spikevax)	mRNA	SARS-CoV-2 spike	COVID-19 prevention	2022	SM-102
mRNA-1345 (mResvia)	mRNA	RSV F protein	RSV — older adults	Phase 3	SM-102
ARO-HSD	siRNA	HSD17B13	Non-alcoholic hepatitis	Phase 2	Proprietary

hATTR: hereditary transthyretin-mediated; RSV: respiratory syncytial virus; TTR: transthyretin; HSD17B13: 17-beta hydroxysteroid dehydrogenase 13.

13. Critical Analysis

The scientific literature on LNP-based nucleic acid delivery reflects a field that has matured considerably but retains several areas of genuine uncertainty and unresolved mechanistic questions that warrant critical appraisal. The pKa-potency relationship, while remarkably consistent across multiple independent research groups, has been derived predominantly from hepatocyte transfection in rodent models and has not been comprehensively validated across extrahepatic cell types or in larger animal species with different endosomal biology. Studies from independent groups have demonstrated that optimal pKa values for lung cell transfection and splenic immune cell targeting may differ substantially from the 6.2 to 6.8 range established for hepatic delivery, suggesting that the pKa-performance relationship is tissue-context-dependent rather than universal [61].

The endosomal escape efficiency of even the most potent clinical LNP formulations is estimated at only 1 to 2% of internalized cargo — a figure that has remained essentially unchanged despite years of intensive ionizable lipid optimization. This raises a fundamental question about whether the incremental improvements in potency achieved by successive ionizable lipid generations reflect genuine improvements in escape efficiency or whether they primarily reflect differences in the amount of LNP internalized per cell. Studies employing quantitative fluorescence imaging have begun to distinguish between uptake efficiency and escape efficiency as independent variables, but the field lacks consensus on standardized assays for their separate measurement. Without this distinction, comparisons of LNP potency across published studies may confound uptake and escape contributions, potentially misdirecting lipid design efforts [62].

The anti-PEG immunogenicity question represents another area of scientific disagreement. While multiple groups have documented generation of anti-PEG IgM and IgG antibodies following LNP administration, the clinical relevance of these antibodies for therapeutic efficacy and safety in repeat-dosed patients has not been prospectively established. Post-marketing pharmacovigilance data from COVID-19 mRNA vaccine recipients have not demonstrated a clear signal of accelerated clearance or reduced immunogenicity with booster doses attributable to anti-PEG antibodies, yet the theoretical concern persists and has motivated research into PEG alternatives including polysarcosine and poly(2-oxazoline) lipids. Resolution of this question requires prospective clinical studies in repeat-dosed patient populations with quantitative anti-PEG antibody monitoring [63].

The in vitro to in vivo translation gap in LNP research is particularly pronounced and has contributed to multiple clinical failures of formulations that appeared highly potent in cell culture transfection assays. The protein corona that forms on LNP surfaces in biological fluids fundamentally alters particle properties, cellular recognition, and organ distribution in ways that cell culture studies cannot recapitulate. Standard in vitro transfection in serum-free or low-serum media conditions systematically overestimates LNP potency relative to in vivo performance. Studies comparing in vitro and in vivo rankings of ionizable lipid libraries have found poor rank-order correlations, underscoring the critical need for physiologically relevant screening assays that incorporate protein corona formation and flow conditions representative of the in vivo environment [64,65].

14. Conclusion

Lipid nanoparticles have traversed the full arc from experimental laboratory concept to globally deployed clinical medicine within the span of two decades, a trajectory that is without precedent in nanomedicine. The four-component LNP platform — ionizable lipid, helper phospholipid, cholesterol, and PEG-lipid — provides a uniquely versatile and tunable architecture that has been successfully adapted for both siRNA gene silencing and mRNA protein expression across a range of disease applications. The clinical approvals of patisiran and the COVID-19 mRNA vaccines, representing different nucleic acid modalities, different disease categories, and different administration routes, demonstrate the breadth and robustness of LNP technology as a pharmaceutical platform.

The ionizable lipid remains the most active area of LNP research, with structure-activity relationships increasingly well understood for hepatic delivery and emerging for extrahepatic tissue targets through SORT technology and rational design approaches. Manufacturing technology based on microfluidic mixing and tangential flow filtration has proven capable of scaling to billions of doses while maintaining formulation quality, establishing that LNP pharmaceutical manufacturing can meet global therapeutic demand.

Unresolved questions regarding endosomal escape mechanisms, anti-PEG immunogenicity in chronically dosed patients, and the translation of in vitro potency data to in vivo performance represent genuine scientific gaps that must be addressed to fulfill the full clinical potential of this platform. The expanding portfolio of clinical programs targeting hereditary metabolic disorders, viral infections, cancer, and protein deficiencies, together with the continued innovation in ionizable lipid chemistry and manufacturing process design, positions LNPs as a defining pharmaceutical technology for the coming decade.

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