STM JOURNALS

Research & Reviews: A Journal of
Toxicology

https://journals.stmjournals.com/rrjot/

ISSN: 2231-3834 (Online)
ISSN: 2349-1264 (Print)
Volume 16, Issue 1, 2026
January–April
DOI (Journal): 10.37591/RRJoT

Review RRJoT

Nanoparticle and Lipid-Based Vaccine Delivery Systems: A Systematic Review of Platforms, Stability, Pharmacokinetics, and Immune Modulation

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Abstract

The rapid evolution of vaccine technologies over the past decade has highlighted the transformative role of nanoparticle (NP) and lipid-based delivery systems in modern immunization practices. These platforms – including polymeric nanoparticles, lipid nanoparticles (LNPs), liposomes, nanoemulsions, and solid lipid nanoparticles (SLNs) – enable enhanced antigen stability, improved cellular uptake, targeted antigen delivery, and potent immune activation. This systematic review critically examines the physicochemical foundations, manufacturing approaches, stability challenges, pharmacokinetic (PK) behavior, and immunomodulatory mechanisms underlying these platforms. Published studies from 2000–2025 were analyzed using PRISMA guidelines. The review synthesizes evidence on formulation performance, biodistribution patterns, antigen release kinetics, and immune response modulation (innate and adaptive). Moreover, comparative insights between NP-based and lipid-based vaccines are provided, along with current applications in mRNA vaccines, viral subunit vaccines, DNA vaccines, and next-generation platforms. Key limitations, toxicity concerns, and future development strategies – including thermostable formulations, targeted delivery, modular vaccine platforms, and scalable continuous-flow manufacturing – are discussed. The review concludes that nanoparticle and lipid systems remain central to the future of vaccinology, providing adaptable, potent, and clinically translatable solutions for emerging infectious threats and precision immunization.

Keywords: Biodistribution, immune modulation, lipid nanoparticles, liposomes, mRNA vaccines, nanoemulsions, nanoparticles, pharmacokinetics, solid lipid nanoparticles, stability, vaccine delivery

INTRODUCTION

Infectious diseases continue to pose a profound burden on global health, despite remarkable advances in immunization science. Traditional vaccines – such as live-attenuated, inactivated, and protein-based platforms – have successfully reduced morbidity and mortality from numerous pathogens. However, their limitations in stability, storage, antigen presentation, and immunogenicity have become increasingly apparent, particularly in the context of rapidly mutating viruses and emerging zoonotic pathogens. The development of nanoparticle and lipid-based vaccine delivery systems represents a paradigm shift, offering unprecedented control over antigen release, protection, targeting, and immune system engagement [1–10].

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Received Date: January 16, 2026
Accepted Date: February 14, 2026
Published Date: April 15, 2026

Citation: Priyanshu Upadhyay, Anand Prakash, Nikhil Vishwakarma. Nanoparticle and Lipid-Based Vaccine Delivery Systems: A Systematic Review of Platforms, Stability, Pharmacokinetics, and Immune Modulation. Research & Reviews: A Journal of Toxicology. 2026; 16(1): 18–36p.

GLOBAL BURDEN OF INFECTIOUS DISEASES

Infectious diseases account for millions of deaths annually, with lower-middle-income countries experiencing the greatest impact. Viral pathogens, such as influenza, HIV, SARS-CoV-2, Zika, and Ebola, continue to challenge conventional vaccine

development pipelines. The need for vaccines that can be rapidly designed, thermally stable, highly immunogenic, and adaptable has never been more urgent [11–16].

The persistence of antimicrobial resistance, emerging pandemics, vector-borne infections, and immunocompromised populations further underline the need for robust and flexible vaccine platforms [17–22].

Rise of Advanced Vaccine Delivery Systems

Traditional vaccines often face challenges, such as:

- Antigen degradation during storage or transport.
- Limited transport across biological barriers.
- Suboptimal induction of mucosal or cellular immunity.
- Requirement of adjuvants to enhance immunogenicity.
- Sensitivity to environmental conditions (e.g., temperature).

To address these gaps, advanced delivery technologies – particularly nanoparticles and lipid carriers – have emerged as essential tools. Their unique physicochemical properties enable:

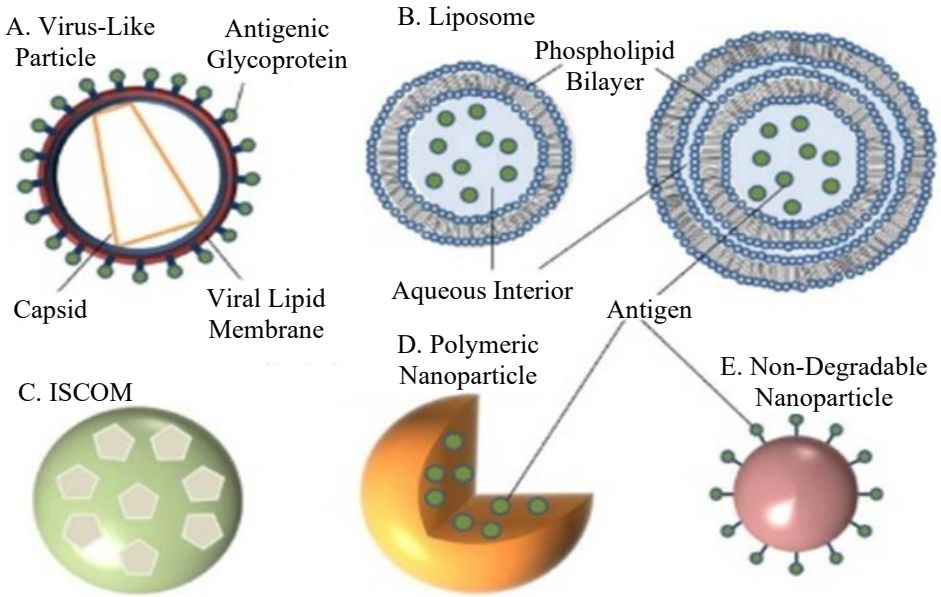
- Protection of antigens against enzymatic and thermal degradation.
- Controlled and sustained antigen release.
- Targeted delivery to antigen-presenting cells (APCs).
- Enhanced activation of innate and adaptive immune pathways.
- Compatibility with modern nucleic-acid-based vaccines (e.g., mRNA, DNA).

Why Nanoparticles and Lipid Systems Are Important

Nanoparticle and lipid-based systems have shown superior performance due to key attributes, such as:

- Nanoscale size enabling lymphatic trafficking.
- High surface area for antigen loading.
- Modifiable surface charge and hydrophobicity.
- Ability to co-deliver antigens and adjuvants.
- Capacity to encapsulate fragile biomolecules (RNA, proteins, peptides).

These systems have become foundational to mRNA vaccines, such as those deployed during the COVID-19 pandemic, showcasing their rapid scalability and clinical potency (Figure 1) [23–25].



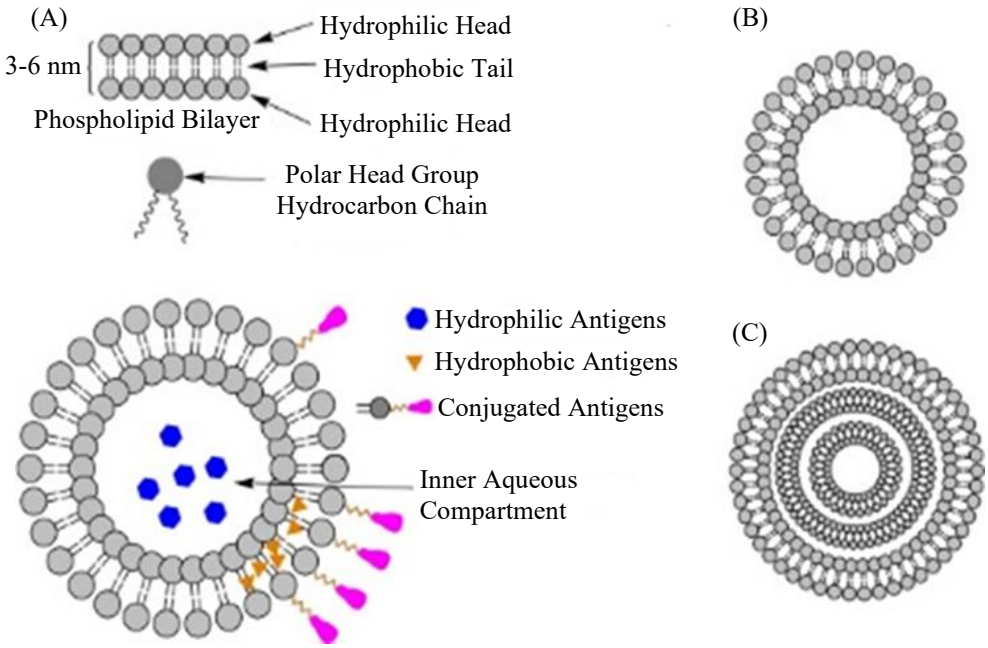


Figure 1. Conceptual overview of NP and lipid-based vaccine carriers.

Need for a Systematic Review on Stability, PK, and Immune Modulation

Although several nanoparticles and lipid technologies exist, a major knowledge gap persists regarding:

- Their comparative stability profiles.
- Pharmacokinetic (PK) behavior including biodistribution and clearance.
- Specific immune-modulatory mechanisms.
- Influence of composition, surface chemistry, and manufacturing methods.
- Challenges in clinical translation, scale-up, and long-term safety.

A systematic, evidence-based review integrating these aspects is essential for guiding future research and regulatory development.

Scope & Objectives of This Review

This review aims to systematically examine:

- Types and platforms of nanoparticle and lipid-based vaccine systems.
- Stability considerations including thermal, physical, and chemical stability.
- Pharmacokinetics and biodistribution dynamics across systems.
- Mechanisms of immune activation and modulation.
- Comparative analysis between nanoparticle vs. lipid-based carriers.
- Real-world applications in approved and emerging vaccines.
- Safety, toxicity, and regulatory aspects.
- Challenges and prospects for next-generation vaccine technologies.

A PRISMA-guided methodology ensures rigor and reproducibility in the selection and evaluation of studies.

OVERVIEW OF NANOPARTICLE-BASED VACCINE DELIVERY SYSTEMS

Nanoparticle (NP)-based vaccine delivery systems have revolutionized modern immunization strategies by providing enhanced antigen stability, targeted immune activation, and versatile formulation capabilities. Their unique physicochemical properties – including nanoscale size, tunable

surface charge, controlled antigen release, and customizable composition – enable efficient delivery of nucleic acids, proteins, peptides, and inactivated pathogens. This chapter provides a structured overview of NP classifications and the mechanistic principles governing their immunological behavior [26–32].

Definition and Classification of Nanoparticle Vaccine Systems

Nanoparticles are submicron-sized colloidal systems (typically 10–500 nm) engineered to encapsulate, adsorb, or conjugate antigens and immunostimulants. Their classification is based primarily using composition, structural organization, biodegradability, and interaction with biological systems (Table 1).

Table 1. Classification of nanoparticle-based vaccine systems.

Parameters	Nanogels	Liposomes	Polymeric nanoparticles	Solid lipid nanoparticles
Size	Nanoscale	Variable, typically 50–100 nm	Variable, typically 10–200 nm	Variable, typically 10–500 nm.
Stability	Good	Moderate	Good	Good.
Drug Loading	High	Moderate	High	High.
Release Mechanism	Controlled release	Variable	Variable	Variable.
Targeting Ability	Can be modified for targeting	Can be modified for targeting	Can be modified for targeting	Can be modified for targeting.
Penetration	Can penetrate biological barriers	Can penetrate biological barriers	Can penetrate biological barriers	Can penetrate biological barriers.
Biocompatibility	Generally biocompatible	Generally biocompatible	Generally biocompatible	Generally biocompatible.
Immunogenicity	Low	Low	Low	Low.
Advantages	High drug loading capacity; controlled release and ability to target specific sites	Lipid bilayer mimics cell membranes; can encapsulate a wide range of drugs	Versatile drug delivery system with tunable properties; potential for surface modification	Enhanced stability and improved drug encapsulation.
Disadvantages	May require additional steps for synthesis and functionalization	Batch-to-batch variability; limited drug loading capacity	Polymeric degradation can lead to loss of stability	May require additional steps for synthesis and functionalization.

Major Classes of Nanoparticles Used in Vaccines

- *Polymeric Nanoparticles*
  - Comprised of biodegradable polymers, such as PLGA, PLA, chitosan, PEG, and alginate.
  - Allow controlled/sustained antigen release.
  - Provide protection against proteolytic and environmental degradation.
- *Inorganic/Metallic Nanoparticles*
  - Include gold nanoparticles (AuNPs), silica NPs, iron oxide NPs.
  - Exhibit unique optical and catalytic properties for antigen display and immune stimulation.
  - High stability and easy surface functionalization.
- *Virus-Like Particles (VLPs)*
  - Self-assembled, non-infectious structures mimicking viral capsids.
  - Naturally immunogenic due to repetitive antigen presentation patterns.
  - Used in HPV and hepatitis B vaccines.
- *Nanogels and Hydrogel Nanoparticles*
  - Hydrophilic polymer networks are capable of high-water uptake.
  - Suitable for mucosal vaccines and protein antigen delivery.
- *Carbon-Based Nanoparticles*
  - Carbon nanotubes, graphene oxide nanoparticles.
  - High loading capacity but require careful toxicity assessment.

Mechanisms of Antigen Delivery and Immune Activation

The effectiveness of NP-based vaccine delivery systems depends on their capacity to facilitate antigen uptake, trafficking, processing, and immune response modulation. Multiple pathways are involved, from initial administration to final immune activation.

Antigen Loading and Presentation

Depending on the platform, antigens may be:

- Encapsulated within the NP core.
- Adsorbed onto the NP surface.
- Covalently conjugated to functional groups.
- Electrostatically bound (e.g., to cationic nanoparticles).

The method of loading directly influences antigen release kinetics and immunogenicity.

Cellular Uptake Pathways

Nanoparticles interact with immune cells through several endocytic mechanisms:

- Clathrin-mediated endocytosis.
- Caveolae-dependent endocytosis.
- Macropinocytosis.
- Phagocytosis (primarily by macrophages and dendritic cells)

Smaller particles (<200 nm) preferentially enter lymphatic channels and accumulate in lymph nodes, while larger particles (>500 nm) are efficiently phagocytosed at injection sites.

Antigen Processing and MHC Presentation

After internalization:

- Endosomal escape allows cytosolic release (critical for DNA/RNA vaccines).
- Antigens are processed through the proteasomal (MHC-I) or endosomal-lysosomal (MHC-II) pathways.
- Resulting peptides are presented on APCs, initiating $CD8^+$ cytotoxic T-cell or $CD4^+$ helper T-cell responses.

Nanoparticles thus enable balanced humoral (antibody) and cellular (T-cell) immunity.

Adjuvant Properties and Immune Modulation

Many nanoparticles inherently possess adjuvant-like features:

- Size and shape influence APC activation.
- Surface charge affects cytokine release and inflammasome activation.
- Patterned/ordered antigen display (as in VLPs) enhances B-cell receptor crosslinking.
- Toll-like receptor (TLR) activators can be co-encapsulated for synergistic immune enhancement.

Chitosan NPs, for example, activate macrophages and dendritic cells via TLR2/4, whereas metallic NPs modulate DC maturation through ROS-mediated pathways.

Advantages of Nanoparticle Platforms in Vaccinology*

- Enhanced antigen stability.
- Efficient mucosal delivery (oral, nasal).
- Targeted lymph node trafficking.
- Controlled release and depot formation.
- Potential for needle-free vaccination.
- Compatibility with nucleic-acid vaccines (DNA, mRNA).
- Reduced need for cold-chain transport in some NP systems.

These characteristics position nanoparticles as a cornerstone of next-generation vaccine technologies.

LIPID-BASED VACCINE DELIVERY SYSTEMS

Lipid-based systems have emerged as one of the most successful platforms for modern vaccines due to their biocompatibility, ability to encapsulate hydrophilic and hydrophobic antigens, and compatibility with nucleic-acid-based delivery (especially mRNA). The unprecedented success of LNP-formulated mRNA vaccines during the COVID-19 pandemic renewed global interest in advanced lipid formulations. This chapter provides an analytical overview of major lipidic vaccine delivery systems, their architectures, manufacturing processes, and mechanistic contributions to immunogenicity [33–37].

Lipid Nanoparticles (LNPs)

Lipid nanoparticles (LNPs) are submicron, self-assembled lipid structures composed of ionizable lipids, helper lipids, phospholipids, cholesterol, and PEG-lipids. Their ability to encapsulate and protect nucleic acids – especially mRNA – makes them one of the most clinically validated vaccine platforms.

Key Components of LNPs

- *Ionizable Lipids*: Facilitate mRNA complexation at low pH and promote endosomal escape.
- *Phospholipids*: Stabilize bilayer structure.
- *Cholesterol*: Enhances membrane fluidity and stability.
- *PEG-Lipids*: Regulate particle size and reduce aggregation.

Functional Advantages

- High transfection efficiency.
- Strong induction of innate and adaptive immunity.
- Lower toxicity compared to permanently cationic lipids.
- Compatibility with scalable microfluidic manufacturing.

Mechanism of Action (Simplified)

LNPs enter cells via endocytosis. Endosomal acidification protonates ionizable lipids. Membrane fusion occurs → mRNA is released into the cytosol. Antigen translation initiates immune activation.

Liposomes

Liposomes are spherical vesicles composed of one or more phospholipid bilayers, capable of encapsulating hydrophilic antigens in the aqueous core and hydrophobic molecules within the bilayer.

Types of Liposomes for Vaccines

- Small unilamellar vesicles (SUVs).
- Large unilamellar vesicles (LUVs).
- Multilamellar vesicles (MLVs).
- Stealth liposomes (PEGylated).

Advantages

- Biocompatible and mimetic of biological membranes.
- Suitable for protein, peptide, DNA, and polysaccharide vaccines.
- Surface can be modified with ligands for targeted APC delivery.

Examples of Liposome Adjuvants

- Cationic liposomal system CAF01 (induces strong Th1/Th17 responses).
- Liposomal AS01 (used in several clinical vaccines).

Solid Lipid Nanoparticles (SLNs)

SLNs are submicron lipid carriers composed of solid lipids (e.g., glyceryl monostearate, stearic acid) that remain solid at room and physiological temperatures.

Advantages
- Improved physical stability.
- Protection of labile antigens.
- Sustained antigen release.
- Potential for mucosal (intranasal/oral) delivery.

Limitations
- Lower loading capacity for hydrophilic antigens.
- Risk of lipid polymorphic transitions affecting antigen stability.

Nanoemulsions and Lipidic Vesicles

Nanoemulsions are thermodynamically unstable but kinetically stable emulsions with droplet sizes in the 20–200 nm range. Oil-in-water (O/W) nanoemulsions are widely used as vaccine adjuvants (Table 2).

Table 2. Comparison of major lipid-based vaccine platforms.

Issue	Advantages of SLNs over liposomes	Advantages of SLNs over polymeric nanoparticles
Avoidance of organic solvents / Preparation and reproducibility	Avoidance of organic solvents when desired. Excellent reproducibility and feasible large-scale production.	Avoidance of organic solvents when desired. Excellent reproducibility and feasible large-scale production with cost-effective high-pressure homogenization method as the preparation method.
Stability	Increased stability of the active ingredients because of the rigid core lipid matrix.	Increased product stability (about 3 years).
Biodegradability	Both liposomes and SLNs are biodegradable.	Lipids of SLNs are physiological and biodegradable, providing better biocompatibility and sterilization. Polymeric nanoparticles may accumulate undesirably in the liver and spleen.
Binding, Entrapment and Release	SLNs provide greater entrapment efficiency for hydrophobic drugs (no aqueous core like liposomes).	Drug delivery is highly site-specific for SLNs, whereas polymeric nanoparticles may show non-specific drug delivery.

Mechanisms of Action
- Enhance antigen dispersion.
- Facilitate uptake by mucosal surfaces.
- Activate innate immune pathways (e.g., via inflammasomes).

Clinical Examples
- Nanoemulsion adjuvant MF59.
- Intranasal nanoemulsion-based vaccines under clinical evaluation.

Manufacturing Technologies

The success of lipid-based vaccines depends heavily on scalable and reproducible manufacturing. Several advanced technologies are utilized:

Microfluidic Mixing
- Enables rapid, controlled mixing of lipids in ethanol with aqueous antigen solution.
- Produces monodisperse LNPs with high encapsulation efficiency.
- Scalable from bench to GMP manufacturing.

High-Pressure Homogenization
- Commonly used for liposomes, nanoemulsions, and SLNs.
- Produces small, uniform droplets or vesicles.
- Useful for heat-stable vaccine formulations.

Ultrasonication
- Effective for reducing vesicle size.
- Suitable for liposomes and nanoemulsions.
- Not ideal for sensitive biological antigens (risk of degradation).

Solvent Evaporation and Thin-Film Hydration
- Traditional method for liposome preparation.
- Allows incorporation of lipophilic molecules.
- Often followed by extrusion to obtain uniform size.

Hot/Cold High-Shear Techniques (for SLNs)
- Combine solid lipids with surfactants at controlled temperatures.
- Enable incorporation of heat-stable antigens.

STABILITY OF NANOPARTICLE AND LIPID SYSTEMS

Stability is a critical factor determining the efficacy, safety, and shelf-life of nanoparticle (NP) and lipid-based vaccine delivery systems. Physicochemical instability can lead to aggregation, phase separation, antigen leakage, hydrolysis, and loss of biological activity – ultimately compromising vaccine potency. This chapter evaluates the major stability determinants and formulation strategies for enhancing the robustness of NP/lipid-based vaccines [38–40].

Physical & Chemical Stability

Nanoparticle and lipid systems are inherently sensitive to environmental and formulation-related stressors, which can cause (Table 3):
- Aggregation / agglomeration.
- Particle size growth (Ostwald ripening).
- Phase separation (e.g., in liposomes and nanoemulsions).
- Chemical degradation including hydrolysis, oxidation, and lipid peroxidation.
- Antigen leakage or degradation.

Table 3. Key physical instability issues.

Instability	Mechanism	Impact
Aggregation	Charge neutralization, inadequate steric stabilization	Reduced cellular uptake, altered PK.
Fusion/Coalescence	Vesicle merging (liposomes, LNPs)	Antigen leakage, loss of structure.
Precipitation	Lipid crystallization (SLNs)	Reduced bioavailability.

Key Chemical Instability Issues
- Lipid peroxidation in LNPs and liposomes.
- Hydrolytic cleavage of phospholipids.
- Polymer degradation in PLGA nanoparticles releasing acidic byproducts.
- mRNA degradation through RNases or hydrolysis.

Thermal Stability

Temperature sensitivity is one of the major challenges in nanocarrier-based vaccine storage. Elevated temperatures accelerate:
- Lipid oxidation.
- Phase transitions.
- Loss of vesicle integrity.
- mRNA degradation in LNPs.

Thermal Behavior of Key Systems
- *LNPs:* Require ultra-cold storage (−20°C to −80°C) for mRNA stability.

- *Liposomes:* Sensitive to phospholipid transition temperatures (Tm); stability depends on lipid composition.
- *SLNs:* More stable at ambient temperatures due to solid lipid matrices.
- *Nanoemulsions:* Temperature fluctuations may cause coalescence or creaming.

Thermostabilization Approaches
- Lyophilization with cryoprotectants.
- Spray drying for thermostable dry powders.
- Incorporation of high-Tm lipids.
- Inclusion of antioxidants (e.g., Vitamin E, BHT).

Surface Charge and Zeta Potential Stability

The zeta potential determines colloidal stability by governing electrostatic repulsion between particles.
- High zeta potential (> ±30 mV) promotes stability.
- Low zeta potential (< ±10 mV) increases aggregation risk.

Influencing Factors
- Ionic strength of the medium.
- pH variations.
- Adsorption of proteins (opsonization).
- PEGylation or surface modifications.

For example, cationic liposomes with high positive charge show strong antigen adsorption but also risk rapid clearance and toxicity. Ionizable lipids in LNPs remain neutral at physiological pH, improving stability and reducing interactions with serum proteins.

Formulation Enhancers (Stabilizers)

Several excipients and formulation adjustments significantly improve stability:

Cryoprotectants and Lyoprotectants
- Trehalose, sucrose, mannitol.
- Protect against freeze–thaw stress and maintain particle integrity.

Antioxidants
- Vitamin E, ascorbic acid, BHT.
- Prevent lipid peroxidation in liposomes and LNPs.

Surfactants/Emulsifiers
- Polysorbates (Tween 20, 80), Span 60.
- Enhance nanoemulsion stability and prevent coalescence.

PEGylation
- Provides steric stabilization.
- Reduces aggregation and opsonization.

High-Transition-Temperature (High-Tm) Lipids
- DSPC, DPPC.
- Improve thermal stability of liposomes/LNPs.

PHARMACOKINETICS (PK) AND BIODISTRIBUTION

Pharmacokinetics (PK) and biodistribution are essential parameters for understanding how nanoparticle and lipid-based vaccine systems behave in vivo after administration. These factors

influence uptake, circulation time, tissue targeting, antigen release, immune activation, and overall vaccine efficacy. Unlike conventional vaccines, nanocarrier-based formulations exhibit unique PK patterns due to their nanoscale size, surface chemistry, and interaction with biological barriers [41].

Absorption and Systemic Uptake

Upon administration – whether intramuscular (IM), subcutaneous (SC), intranasal (IN), or oral – nanocarriers undergo physicochemical interactions that determine their absorption profile.

Key Determinants of Absorption

- *Particle Size*: Small particles (<200 nm) readily enter lymphatic vessels. Larger particles (>500 nm) remain localized, forming depots for slow release.
- *Surface Charge*: Cationic particles interact more strongly with cell membranes, enhancing uptake but risking cytotoxicity. Neutral particles show prolonged retention with lower immunogenicity.
- *Hydrophobicity*: Affects membrane permeability and cellular internalization.

Injection Site Dynamics

For IM/SC administration:

- Nanoparticles accumulate in the extracellular matrix.
- Macrophages and dendritic cells (DCs) internalize particles.
- Lymphatic drainage transports particles to lymph nodes, where antigen presentation is initiated.

Distribution and Lymphatic Targeting

Lymph nodes are central to effective vaccine responses. Nanoparticles and LNPs with optimal size and surface profiles demonstrate enhanced targeting to lymphoid tissues (Table 4).

Table 4. Biodistribution profiles of various platforms.

System	Primary distribution sites	Notes
LNPs	Injection site → lymph nodes → liver	PEGylation reduces hepatic uptake; ionizable lipids modulate circulation.
Liposomes	Lymph nodes, spleen; some liver accumulation	High variability is based on charge and lipid composition.
Polymeric NPs (PLGA)	Lymph nodes, spleen, liver	Slow degradation prolongs antigen presentation.
SLNs	Reticuloendothelial system (Res), mucosal surfaces	Good potential for oral/nasal delivery.
Nano emulsions	Mucosa → lymph nodes	Ideal for intranasal vaccines.

Liver Accumulation in LNPs

Due to protein corona formation and ApoE-mediated uptake, LNPs commonly accumulate in the liver. This is beneficial for some genetic therapies but requires careful control for vaccines.

Metabolism and Biotransformation

Nanocarriers undergo metabolic processes depending on their chemical nature:

- *Lipid Nanoparticles:* Ionizable lipids are metabolized by hepatic lipid-processing enzymes. PEG-lipids dissociate over time, influencing circulation half-life.
- *Liposomes:* Broken down by phospholipases, releasing constituent lipids for natural metabolic pathways.
- *Polymeric Nanoparticles:* PLGA degrades into lactic and glycolic acid via hydrolysis. Degradation rate influences antigen release duration.
- *SLNs:* Lipids undergo enzymatic lipolysis. Hydrolytic breakdown is slower due to solid matrix.

Elimination and Clearance

Clearance pathways differ widely among nanocarrier systems:

- *Renal Clearance:* Particles < 5–10 nm (rare for vaccines) eliminated through glomerular filtration.
- *Hepatobiliary Clearance:* LNPs, liposomes, and polymeric NPs (>100 nm) are taken up by Kupffer cells and cleared via bile.
- *Phagocytic Clearance (RES System):* Macrophages in liver, spleen, and lymph nodes engulf nanoparticles. Surface modification (e.g., PEGylation) reduces opsonization and RES uptake.
- *Mucociliary Clearance (for intranasal vaccines):* Nanoemulsions and small lipid vesicles may be cleared rapidly unless mucoadhesive agents are incorporated.

Pharmacokinetic Parameters in Nanocarrier Vaccines

Nanocarrier-based vaccines exhibit PK profiles distinct from small drugs. The following parameters are modified (Table 5):

- *Cmax:* Lower than free antigens due to slow release.
- *Tmax:* Delayed due to depot formation.
- *AUC:* Higher, indicating sustained antigen presence.
- *t1/2:* Extended due to controlled release and reduced enzymatic degradation.
- *Volume of Distribution:* Modified by surface charge and protein corona formation.

Depot-release formulations show a biphasic PK curve. LNPs display rapid distribution followed by slower clearance.

Table 5. Comparative PK performance among major platforms.

Platform	Circulation half-life	Lymphatic targeting	Clearance mechanism
LNPs	Moderate	High	Hepatobiliary.
Liposomes (PEGylated)	Long	Moderate	RES + slow enzymatic cleavage.
Polymeric NPs	Long (hours–days)	High	Hydrolysis + phagocytosis.
SLNs	Moderate	Good	Enzymatic lipid degradation.
Nanoemulsions	Short	High (mucosal)	Mucociliary + RES.

IMMUNE MODULATION MECHANISMS

Nanoparticle (NP) and lipid-based vaccine delivery systems do more than transport antigens—they actively shape the nature, magnitude, and duration of immune responses. Their physicochemical attributes (size, shape, charge, composition, and surface ligands) determine how they interact with immune cells, pattern recognition receptors (PRRs), lymphatic pathways, and signaling cascades. This chapter outlines how these systems modulate innate and adaptive immunity to enhance vaccine performance [42].

Interaction with Innate Immune System

Innate immune activation is the essential first step for a strong and durable adaptive response. Nanocarriers can directly engage PRRs, recruit antigen-presenting cells (APCs), and stimulate inflammatory mediators.

Key Mechanisms

Pattern Recognition Receptor (PRR) Activation

Nanocarriers can activate:

- Toll-like receptors (TLRs) – especially TLR3, TLR7/8 for RNA-based vaccines.
- NOD-like receptors (NLRs) – inflammasome activation (e.g., NLRP3).
- C-type lectin receptors (CLRs) – dendritic cell (DC) targeting by mannose-modified NPs.

Ionizable lipids in LNPs can stimulate transient innate responses via interferon pathways, enhancing adjuvanticity.

Inflammasome Activation

Certain particles induce controlled inflammation by activating:

- NLRP3 inflammasome → IL-1β, IL-18 release.

This enhances DC maturation and antigen presentation.

Complement Activation

Surface charge and phospholipid composition influence complement system engagement, facilitating opsonization and uptake by phagocytes.

Antigen Uptake and Processing

Nanocarriers significantly enhance the efficiency of antigen capture by APCs.

Mechanistic Highlights

- *Endocytosis Pathways:* Clathrin-mediated, caveolae-mediated, macropinocytosis, phagocytosis.
- *Endosomal Escape:* Crucial in mRNA/LNP vaccines.
- *Cross-Presentation:* NP encapsulation promotes MHC-I antigen loading, boosting $CD8^+$ T-cell responses.

LNPs, due to their ionizable lipid content, are exceptionally efficient at endosomal escape, enabling cytosolic release of mRNA.

Modulation of Adaptive Immunity

Adaptive immunity consists of humoral (B-cell mediated) and cellular (T-cell mediated) responses. Nanocarriers influence both pathways.

B-Cell Activation and Antibody Production

Nanoparticles facilitate:

- Repetitive antigen display → B-cell receptor (BCR) crosslinking.
- Enhanced germinal center formation.
- Superior class-switching (IgG1/IgG2a, IgA for mucosal vaccines).

Virus-like particles (VLPs) are particularly effective at inducing strong antibody responses due to their multivalent antigen arrangement.

T-Cell Activation

Nanocarriers promote:

- *$CD4^+$ T-Helper Responses:* (Th1, Th2, Th17 depending on adjuvants/surface chemistry).
- *$CD8^+$ Cytotoxic T Lymphocyte (CTL) Activation:* through cross-presentation.

For instance, cationic liposomes induce strong Th1/Th17 responses, whereas polymeric nanoparticles facilitate durable CTL memory due to sustained antigen release, and mRNA-LNP vaccines promote robust $CD4^+$ TfH and $CD8^+$ responses.

Cytokine and Chemokine Modulation

Nanocarriers induce specific cytokine profiles that influence the nature of immunity (Table 6).

Table 6. Typical cytokine patterns.

Nanocarrier Type	Dominant cytokines	Immunological effect
LNPs (mRNA vaccines)	IFN-α, IFN-β, IL-6	Strong innate activation, T-cell priming.
Cationic liposomes	IL-12, TNF-α	Th1 polarization.
SLNs	IL-6, IL-1β	Mild innate activation.
Polymeric NPs	IL-2, IFN-γ	T-cell expansion and memory.

Lymph Node Targeting and Germinal Center Responses

Lymph nodes are immunological hubs where antigen presentation and T–B cell interactions occur. Nanoparticles sized between 20–200 nm efficiently reach lymphatic tissues.

Mechanisms Contributing to Lymph Node Immune Activation

- Enhanced APC recruitment.
- Sustained antigen presence.
- Improved follicular helper T-cell (TfH) activation.
- Prolonged germinal center (GC) reactions → high-affinity antibodies.

Immune Polarization (Th1/Th2/Th17) by Nanocarriers

The physicochemical nature of nanocarriers dictates immune polarization:

- *Th1 Responses*: Promoted by cationic liposomes, LNPs, polymeric NPs; characterized by IFN-γ, IL-12; supports antiviral and intracellular pathogen protection.
- *Th2 Responses*: Favored by neutral or anionic liposomes; involves IL-4, IL-5, IL-13; ideal for allergy or parasitic vaccines.
- *Th17 Responses*: Characteristic of some liposomal adjuvant systems (e.g., CAF01); critical for mucosal immunity.

Adjuvant Synergy in Nanocarrier Systems

Nanocarriers can co-deliver adjuvants, such as:

- CpG (TLR9 agonist).
- MPLA (TLR4 agonist).
- Poly(I:C) (TLR3 agonist).
- QS-21 (saponin adjuvant), and aluminum salts (hybrid nano/alum formulations).

This synergistic delivery enhances tailoring of immune responses toward specific pathogens.

APPLICATIONS IN CURRENT AND EMERGING VACCINES

Nanoparticle and lipid-based vaccine delivery systems have moved from experimental platforms to established components of modern vaccinology. Their ability to protect fragile biomolecules, enhance immunogenicity, and support rapid vaccine development has enabled breakthroughs across infectious diseases, oncology, and emerging viral threats. This chapter highlights the current applications, regulatory successes, and future innovations driven by NP and lipid systems [43].

Applications in Licensed and Widely Deployed Vaccines

The most significant global demonstration of nanocarrier utility was seen during the COVID-19 pandemic. Lipid nanoparticles (LNPs) enabled the efficient delivery of mRNA encoding viral antigens, paving the way for ultra-rapid vaccine development and global immunization.

Role of LNPs in mRNA Vaccines

- Protect mRNA from enzymatic degradation.
- Facilitate cellular uptake and endosomal escape.
- Enable high-yield antigen expression in host cells.
- Induce strong innate and adaptive immunity.

Advantages Demonstrated in Real-World Deployment

- Rapid production scalability (microfluidics-based LNP manufacturing).
- Ability to quickly redesign mRNA for emerging variants.
- Potent humoral and cellular responses.
- Demonstrated safety and tolerability in large populations.

LNP technology thus established itself as a cornerstone for future rapid-response vaccine platforms.

Nanoparticle-Based Platforms in Existing Vaccines

Several currently approved vaccines or vaccine candidates employ nanoparticle components:

- *Virus-Like Particles (VLPs):* Used successfully for hepatitis B and human papillomavirus (HPV) vaccines. Highly immunogenic due to repetitive antigen arrangement. Do not contain genetic material → non-infectious and safe.
- *Alum–Nanoparticle Hybrids:* Traditional aluminum salts combined with nanoscale antigens improve adsorption and depot effect. Enhance dendritic cell activation and cytokine production.
- *Polymeric Nanoparticles:* Being evaluated in influenza, malaria, tuberculosis, and HIV vaccine trials. Show promise in sustained antigen release and CTL activation.

Applications in Next-Generation Vaccines

With advances in genomics and synthetic biology, nanocarrier-based systems are enabling entirely new categories of vaccines.

DNA Vaccines Delivered by Nanoparticles

- Cationic polymeric NPs improve plasmid DNA uptake.
- Enhanced nuclear localization compared to naked DNA.
- Applications: Zika, dengue, cancer neoantigen vaccines.

Protein/Peptide Nanoparticle Vaccines

- PLGA and chitosan nanoparticles stabilize fragile peptides.
- Promote sustained release and stronger T-cell responses.
- Useful for malaria, HIV, tuberculosis, and helminthic infections.

Self-Assembling Nanoparticle Vaccines

- Designed antigen arrays assembled into nanoscale structures.
- Induce strong germinal center responses.
- Ideal for rapidly mutating viruses like influenza or coronaviruses.

Applications in Mucosal Vaccination

Mucosal vaccines aim to generate protective immunity at entry sites of pathogens (respiratory, gastrointestinal, urogenital). Nanoemulsions, SLNs, and mucoadhesive polymeric nanoparticles offer unique advantages.

Key Benefits

- Needle-free administration.
- Induction of secretory IgA (sIgA).
- Strong mucosal and systemic immunity.
- Improved patient compliance.

Prominent applications include intranasal influenza vaccines using nanoemulsions, oral SLN-based vaccines for enteric pathogens, and mucoadhesive chitosan NPs for respiratory diseases.

Applications in Cancer Vaccines

Nanoparticle platforms are increasingly integrated into therapeutic cancer vaccines due to their ability to deliver tumor-associated antigens (TAAs) and adjuvants to antigen-presenting cells.

Advantages in Oncology

- Enhanced lymph node targeting.

- Potent $CD8^+$ cytotoxic T-cell activation.
- Co-delivery of antigens + immune stimulants (CpG, Poly(I:C)).

Personalization using mRNA or neoantigen peptides. Examples under clinical evaluation include polymeric NP-based melanoma vaccines and LNP-formulated mRNA cancer vaccines.

Applications for Emerging and Re-Emerging Viral Threats

Nanocarrier-based platforms are extensively explored for:

- *Zika and Dengue:* LNP-mRNA and nanoemulsion vaccines.
- *Ebola and Marburg Viruses:* VLP and polymeric NP vaccines.
- *RSV:* Lipid and protein nanoparticle formulations.
- *Avian Influenza:* Self-assembling nanoparticles displaying HA antigens.
- *Tuberculosis:* Liposomal adjuvant systems (CAF01).

Their flexibility allows rapid antigen redesign and scalable manufacturing – critical for outbreak preparedness.

Applications in Veterinary Vaccines

Nanoparticles and lipid systems are increasingly used in veterinary immunization for:

- Foot-and-mouth disease (FMD).
- Newcastle disease.
- Swine influenza.
- Bovine respiratory diseases.

These systems improve stability under field conditions and reduce the required vaccine dose.

CHALLENGES, LIMITATIONS, AND FUTURE DIRECTIONS

Although nanoparticle (NP) and lipid-based vaccine delivery systems have transformed vaccinology, several scientific, regulatory, and logistical challenges still restrict their global utilization. This chapter outlines key limitations and emerging strategies that will shape future development.

Scientific and Technical Challenges

Stability and Cold-Chain Dependence

Lipid nanoparticles (especially mRNA-LNP systems) require ultra-cold storage (−20°C to −80°C) to maintain structural integrity and prevent mRNA degradation. This severely limits distribution in low-resource settings.

Scale-Up and Manufacturing Complexity

- Microfluidic mixing for LNPs requires specialized equipment.
- Polymeric nanoparticle synthesis (nanoprecipitation, solvent evaporation) must maintain batch-to-batch uniformity.
- VLP production demands-controlled expression systems and purification steps.

Limited Thermostability

Many lipidic systems undergo phase transitions, oxidation, or hydrolysis at elevated temperatures, impacting antigen retention and immunogenicity.

Incomplete Understanding of Immune Interactions

Nanoparticle–immune system interactions (e.g., PRR activation, protein corona formation, complement activation) are not fully mapped. These unknowns may influence reactogenicity and long-term immunity.

Safety and Regulatory Barriers

Need for Long-Term Toxicity Data

- Inorganic nanoparticles may accumulate in organs.
- PEG-related hypersensitivity necessitates alternative stabilizers.
- Polymeric NP degradation by-products may influence tissue microenvironments.

Regulatory Framework Gaps

Current vaccine regulatory guidelines are designed around traditional formulations. Nanocarrier-specific standards are evolving but vary between agencies (FDA, EMA, WHO).

Analytical Limitations

Advanced characterization tools (Cryo-EM, DLS, AFM, HPLC-RNA integrity assays) are essential but expensive and not widely available.

Economic and Logistical Challenges

- High cost of ionizable lipids and proprietary LNP synthesis technologies.
- Requirement for refrigerated or deep-cold transportation.
- Patent restrictions limiting widespread manufacturing in low-income regions.

Future Directions in Nanocarrier-Based Vaccines

Thermostable Vaccine Formulations

Innovations include:

- Lyophilized LNPs.
- Spray-dried polymeric nanoparticles.
- Thermostable SLN-based oral vaccines.
- Hydrated nanogels with high-temperature resilience.

These can drastically reduce cold-chain dependency.

Next-Generation Nanomaterials

- Polymer–lipid hybrid nanoparticles.
- Bio-inspired nanocarriers (exosomes, membrane-coated NPs).
- Self-assembling nanoparticle scaffolds.
- Smart nanocarriers are responsive to pH, enzymes, or temperature.

Personalized Vaccines

mRNA-LNP and peptide–nanoparticle vaccines enable:

- Patient-specific cancer vaccines.
- Rapid antigen redesign for emerging pathogens.
- Customized dosing based on immune profiling.

Mucosal and Needle-Free Vaccination

Nanoemulsions, SLNs, and mucoadhesive polymers will enable:

- Intranasal influenza and RSV vaccines.
- Oral vaccines for enteric pathogens.
- Inhalable cancer vaccines.

AI and Computational Tools

Machine learning will optimize:

- Nanoparticle composition.
- Antigen design.

- PK/PD prediction.
- Toxicity forecasting.

Global Decentralized Manufacturing

Portable microfluidic systems will support on-site vaccine production during outbreaks.

CONCLUSION

Nanoparticle and lipid-based vaccine delivery systems have revolutionized the landscape of modern immunization. From polymeric nanoparticles to lipid nanoparticles and nanoemulsions, these platforms provide unique advantages in antigen stability, targeted delivery, controlled release, and immune modulation. Their success – especially LNP-formulated mRNA vaccines – demonstrates the immense potential of nanotechnologies in rapid-response vaccine development, pandemic preparedness, and precision medicine.

However, challenges, such as cold-chain requirements, limited thermostability, regulatory gaps, and incomplete immune interaction data, remain significant. Continued innovation in material engineering, computational modeling, personalized vaccine platforms, and thermostable formulations will enable broader global adoption. Ultimately, nanocarrier-based vaccines represent a transformative shift toward safe, potent, adaptable, and scalable solutions capable of addressing current and future infectious disease threats.

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