WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 21, 27-57. Review Article ISSN 2277–7105

ADVANCED LIPOSOMAL CO-DELIVERY STRATEGIES FOR SYNERGISTIC ANTI-INFLAMMATORY THERAPY

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Article Received on 02 Oct. 2026,
Article Revised on 22 Oct. 2026,
Article Published on 01 Nov. 2026,

https://doi.org/10.5281/zenodo.23756456

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How to cite this Article: Pankaj Kumar*, Prem Prasad, Dr. Sanjay Kumar Kushwaha (2026). Advanced Liposomal Co-Delivery Strategies For Synergistic Anti-Inflammatory Therapy. World Journal of Pharmaceutical Research, 15(21), 27–57

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ABSTRACT

Inflammation is a complex physiological response associated with the progression of numerous acute and chronic diseases, including rheumatoid arthritis, inflammatory bowel disease, psoriasis, pulmonary disorders, neurodegenerative conditions, and cardiovascular diseases. Although conventional anti-inflammatory therapies such as nonsteroidal anti-inflammatory drugs (NSAIDs): corticosteroids, and immunosuppressive agents are widely used, their long-term administration is often limited by poor bioavailability, rapid metabolism, systemic toxicity, non-specific tissue distribution, and severe adverse effects. To overcome these limitations, liposomal nanocarrier systems have emerged as promising drug delivery platforms due to their biocompatibility, biodegradability, controlled release behavior, and targeting potential. Among these systems, dual drug-loaded liposomes have gained considerable attentionfor their ability to simultaneously deliver two therapeutic agents with complementary mechanisms of action, thereby enhancing synergistic anti-inflammatory efficacy while reducing toxicity and dosing frequency. This review highlights recent advances in dual drug-loaded liposomal systems developed for synergistic anti-inflammatory therapy. It discusses the structural organization, composition, classification, and formulation strategies of liposomes, including thin-film hydration, reverse-phase evaporation, ethanol injection, and active loading methods. Important physicochemical characterization parameters such as particle size, zeta potential, entrapment efficiency, drug release behavior, and stability are also summarized. Particular emphasis is placed on the co-delivery of synthetic anti-inflammatory drugs with natural

bioactive compounds, antioxidants, immunomodulators, and nucleic acid therapeutics for simultaneous modulation of inflammatory pathways including NF-κB, MAPK, COX-2, cytokine signaling, and oxidative stress-mediated mechanisms. Furthermore, recent in vitro and in vivo studies demonstrating enhanced therapeutic efficacy, controlled drug release, reduced inflammatory cytokine production, and targeted accumulation at inflamed tissues are critically discussed. The review also addresses current challenges associated with large-scale manufacturing, formulation stability, regulatory approval, and clinical translation. Overall, dual drug-loaded liposomal systems represent highly promising nanotherapeutic platforms for the development of safer, more effective, and targeted anti-inflammatory therapies in the future.

1. INTRODUCTION

Inflammation is a complex physiological and immunological response initiated by the body in reaction to harmful stimuli such as pathogens, tissue injury, toxins, allergens, or autoimmune reactions. It serves as a protective mechanism aimed at eliminating injurious agents and restoring tissue homeostasis.[1] The inflammatory response involves a coordinated interaction between immune cells, cytokines, chemokines, adhesion molecules, and inflammatory mediators including prostaglandins, leukotrienes, tumor necrosis factor-alpha (TNF-α): interleukins, and reactive oxygen species (ROS). Acute inflammation is generally beneficial and self-limiting; however, chronic or uncontrolled inflammation contributes significantly to the pathogenesis of numerous diseases, including rheumatoid arthritis, inflammatory bowel disease, psoriasis, asthma, cardiovascular disorders, neurodegenerative diseases, diabetes mellitus, and cancer.[2]

The growing prevalence of chronic inflammatory disorders has created a substantial global healthcare burden and highlights the need for more effective and safer therapeutic strategies. Conventional anti-inflammatory therapies primarily include nonsteroidal anti-inflammatory drugs (NSAIDs): corticosteroids, immunosuppressive agents, and biologics. These therapeutic agents exert their effects through inhibition of inflammatory signaling pathways, suppression of cytokine production, or modulation of immune responses.[3] NSAIDs mainly inhibit cyclooxygenase (COX) enzymes and reduce prostaglandin synthesis, whereas corticosteroids suppress multiple inflammatory genes through modulation of nuclear factor-kappa B (NF-κB) and other transcription factors.[4]

Despite their clinical effectiveness, conventional anti-inflammatory therapies possess several limitations that restrict long-term use and therapeutic efficiency.[5] Prolonged administration of NSAIDs is frequently associated with gastrointestinal ulceration, renal toxicity, cardiovascular complications, and hepatotoxicity. Corticosteroids may cause immunosuppression, osteoporosis, metabolic disturbances, adrenal suppression, and increased susceptibility to infections. Similarly, biologic therapies and immunosuppressive agents often exhibit high treatment costs, poor patient compliance, systemic adverse effects, and increased risk of opportunistic infections.[6]

Another major challenge associated with conventional anti-inflammatory drugs is poor pharmacokinetic behavior, including limited aqueous solubility, low bioavailability, rapid metabolism, short circulation half-life, and non-specific tissue distribution. Many anti-inflammatory agents fail to achieve adequate therapeutic concentrations at inflamed tissues while simultaneously affecting healthy tissues, thereby increasing systemic toxicity. Furthermore, repeated dosing and fluctuating plasma drug concentrations may compromise therapeutic outcomes and patient adherence.[7]

To overcome these limitations, advanced nanocarrier-based drug delivery systems have gained considerable attention in recent years. Among these systems, liposomes have emerged as highly promising nano vesicular carriers for anti-inflammatory therapy due to their unique structural characteristics, biocompatibility, biodegradability, and ability to encapsulate both hydrophilic and lipophilic drugs. Liposomes are spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core. Their structural similarity to biological membranes enables efficient interaction with cells and facilitates targeted drug delivery.[8]

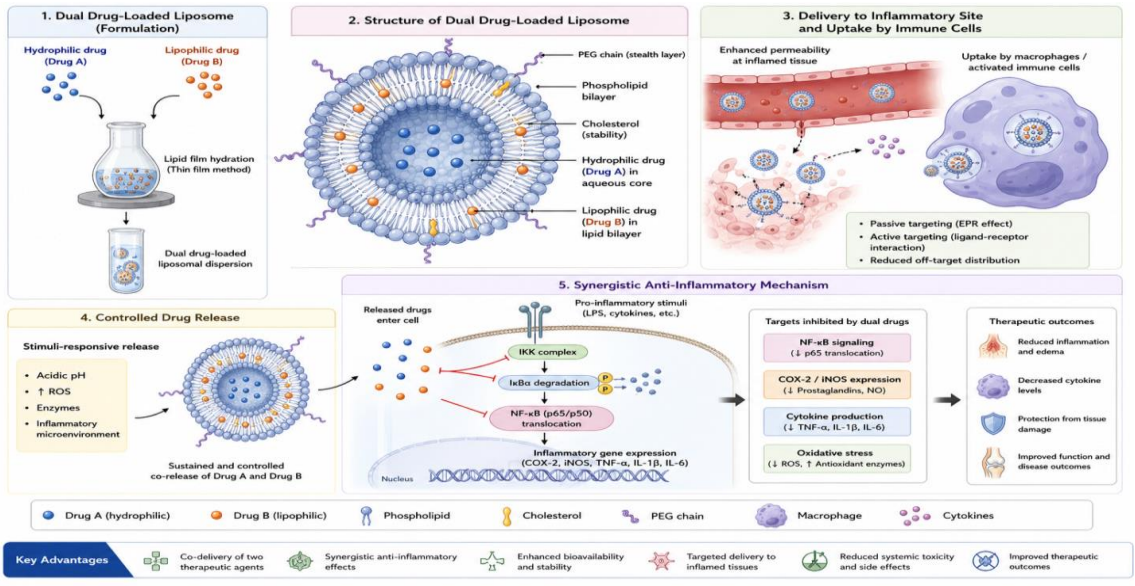
Liposomal drug delivery systems offer several advantages over conventional formulations, including improved drug solubility, enhanced bioavailability, prolonged circulation time, controlled drug release, reduced systemic toxicity, and targeted accumulation at inflamed tissues. Surface modification strategies such as PEGylation, ligand conjugation, and stimuli-responsive functionalization further enhance liposomal stability and targeting efficiency. Liposomes can passively accumulate at inflamed sites through enhanced vascular permeability and inflammatory microenvironment-associated retention mechanisms.[9]

Recently, dual drug-loaded liposomal systems have attracted increasing scientific interest for anti-inflammatory applications. These systems involve simultaneous encapsulation of two

therapeutic agents within a single liposomal carrier to achieve synergistic therapeutic effects, improved pharmacokinetic profiles, reduced drug resistance, and enhanced treatment efficacy. The combined delivery of anti-inflammatory drugs, antioxidants, immunomodulators, natural bioactive compounds, or nucleic acid therapeutics can target multiple inflammatory pathways simultaneously and improve overall therapeutic outcomes.[10]

Dual drug-loaded liposomes provide several advantages, including synchronized drug delivery, controlled release kinetics, reduced dosing frequency, and minimized adverse effects. Co-encapsulation strategies may also improve the stability of labile drugs and enable combination therapy with lower individual drug doses, thereby reducing toxicity. These multifunctional liposomal systems have shown promising therapeutic potential in various inflammatory disorders, including rheumatoid arthritis, psoriasis, inflammatory bowel disease, pulmonary inflammation, and neuroinflammatory conditions.[11]

The present review aims to comprehensively discuss recent trends and advances in dual drug-loaded liposomal systems for anti-inflammatory applications. The review focuses on the structural characteristics of liposomes, formulation strategies, dual drug loading approaches, mechanisms of anti-inflammatory action, physicochemical characterization, therapeutic applications, and recent research developments.[12] Furthermore, current challenges associated with formulation stability, scale-up, clinical translation, and regulatory considerations are critically addressed along with future perspectives for the development of advanced liposomal nanotherapeutics in inflammatory disease management.[11]



2. Liposomal Systems for Dual Drug Delivery

Liposomal drug delivery systems have emerged as versatile and highly efficient nanocarriers for the co-delivery of multiple therapeutic agents in the treatment of inflammatory disorders. Their unique vesicular architecture, biocompatibility, and ability to encapsulate both hydrophilic and hydrophobic compounds make them ideal candidates for combination therapy. In recent years, dual drug-loaded liposomal systems have attracted substantial attention due to their capability to simultaneously target multiple inflammatory pathways, enhance therapeutic efficacy, reduce systemic toxicity, and improve pharmacokinetic performance.[10]

The co-encapsulation of two therapeutic agents within a single liposomal carrier enables synchronized delivery and controlled release at inflamed tissues, thereby maximizing synergistic therapeutic effects. Dual drug-loaded liposomes have been extensively investigated for the combined delivery of anti-inflammatory drugs, corticosteroids, antioxidants, immunomodulators, phytochemicals, nucleic acids, and biological agents.[10]

2.1 Structure and Composition of Liposomes

Liposomes are spherical vesicular systems composed primarily of phospholipid bilayers surrounding one or more aqueous compartments. The amphiphilic nature of phospholipids enables spontaneous self-assembly into bilayer structures in aqueous environments. Hydrophilic drugs are generally encapsulated within the aqueous core, whereas lipophilic drugs are incorporated into the phospholipid bilayer.[13]

The structural organization of liposomes closely resembles biological cell membranes, which contributes to their excellent biocompatibility and reduced immunogenicity. Liposomal systems generally range in size from nanometers to micrometers depending on formulation composition and preparation methods.[12]

The major components of liposomal formulations include.

2.1.1 Phospholipids

Phospholipids form the primary structural framework of liposomes. Commonly used phospholipids include, Phosphatidylcholine, Phosphatidylserine, Phosphatidylethanolamine, Sphingomyelin, Hydrogenated soy phosphatidylcholine (HSPC)These phospholipids influence membrane fluidity, permeability, stability, and drug encapsulation efficiency.[14]

2.1.2 Cholesterol

Cholesterol is frequently incorporated into liposomal bilayers to enhance membrane rigidity and stability. It reduces membrane permeability, minimizes drug leakage, and prolongs circulation time by stabilizing phospholipid packing.[15]

2.1.3 Surface Modifiers

Surface modification strategies are commonly employed to improve liposomal performance. Polyethylene glycol (PEG) is widely used to produce stealth liposomes with prolonged systemic circulation and reduced uptake by the reticuloendothelial system (RES). Ligands such as antibodies, peptides, aptamers, and carbohydrates may also be conjugated to liposomal surfaces for active targeting of inflamed tissues.[16]

2.1.4 Encapsulated Therapeutic Agents

Dual drug-loaded liposomes may contain combinations of, NSAIDs and corticosteroids, Anti-inflammatory drugs and antioxidants, Synthetic drugs and natural phytochemicals, small molecules and nucleic acids, Immunomodulators and biologics, The encapsulation efficiency and localization of drugs within the liposome depend on their physicochemical properties such as solubility, polarity, and molecular weight.[17]

2.2 Types of Liposomal Formulations

Liposomal systems can be classified based on vesicle size, lamellarity, composition, surface characteristics, and functional modifications. Various liposomal formulations have been developed to optimize dual drug delivery and anti-inflammatory performance.[12]

2.2.1 Conventional Liposomes

Conventional liposomes consist of phospholipid bilayers with or without cholesterol. They are capable of encapsulating both hydrophilic and lipophilic drugs but may exhibit limited circulation stability due to rapid recognition and clearance by macrophages.[18]

2.2.2 PEGylated (Stealth) Liposomes

PEGylated liposomes contain polyethylene glycol chains attached to the liposomal surface. PEGylation provides steric stabilization, reduces opsonization, and prolongs blood circulation time. These liposomes demonstrate improved accumulation at inflamed tissues through enhanced permeability and retention (EPR)-like inflammatory targeting mechanisms.[19]

2.2.3 Targeted Liposomes

Targeted liposomes are surface-functionalized with ligands capable of selectively binding to receptors overexpressed at inflamed sites. These systems improve site-specific drug delivery and reduce systemic toxicity.[20] Examples of targeting ligands include: Antibodies, Folate, Hyaluronic acid, Peptides, Mannose, Transferrin.

2.2.4 Cationic Liposomes

Cationic liposomes possess positively charged surfaces that facilitate interaction with negatively charged cell membranes and nucleic acids. These systems are particularly useful for gene delivery and immunomodulatory applications.[21]

2.2.5 Stimuli-Responsive Liposomes

Stimuli-responsive liposomes are designed to release drugs in response to environmental triggers such as, pH changes, Temperature, Reactive oxygen species, Enzymes Ultrasound, Magnetic fields. Inflamed tissues often exhibit acidic pH, oxidative stress, and elevated enzyme activity, which can be exploited for site-specific drug release.[22]

2.2.6 Multilamellar and Unilamellar Liposomes

Based on lamellarity, liposomes may be classified into, Small unilamellar vesicles (SUVs): Large unilamellar vesicles (LUVs): Multilamellar vesicles (MLVs): These structural variations influence drug loading capacity, release kinetics, and stability.[23]

2.3 Advantages of Dual Drug-Loaded Liposomes

Dual drug-loaded liposomal systems offer numerous therapeutic and pharmacological advantages compared to single-drug formulations and conventional drug delivery approaches.[24]

2.3.1 Synergistic Therapeutic Activity

Simultaneous delivery of two therapeutic agents enables targeting of multiple inflammatory pathways, resulting in enhanced anti-inflammatory efficacy and synergistic therapeutic responses.[25]

2.3.2 Controlled and Sustained Drug Release

Liposomal carriers provide controlled release of encapsulated drugs over prolonged periods, maintaining therapeutic drug concentrations while reducing dosing frequency.[26]

2.3.3 Improved Drug Stability

Encapsulation within liposomal bilayers protects drugs from enzymatic degradation, oxidation, hydrolysis, and premature metabolism.[27]

2.3.4 Enhanced Bioavailability

Liposomes improve the solubility and absorption of poorly water-soluble anti-inflammatory drugs, thereby increasing bioavailability and therapeutic effectiveness.[28]

2.3.5 Reduced Systemic Toxicity

Targeted and localized delivery minimizes drug exposure to healthy tissues, reducing systemic adverse effects commonly associated with conventional anti-inflammatory therapy.[29]

2.3.6 Simultaneous Delivery of Hydrophilic and Lipophilic Drugs

The unique bilayer structure of liposomes allows co-encapsulation of drugs with different solubility characteristics within a single carrier system.[21]

2.3.7 Improved Pharmacokinetic Profiles

Dual drug-loaded liposomes can prolong circulation time, reduce rapid drug clearance, and improve tissue retention.[30]

2.3.8 Enhanced Patient Compliance

Sustained drug release and reduced dosing frequency contribute to improved patient adherence and therapeutic outcomes.[31]

2.4 Mechanisms Underlying Synergistic Therapeutic Effects

The therapeutic superiority of dual drug-loaded liposomal systems is largely attributed to synergistic interactions between co-encapsulated agents and their ability to modulate multiple inflammatory pathways simultaneously.[32] Inflammation involves complex signaling networks including, nuclear factor-kappa B (NF-κB): Cyclooxygenase (COX) pathways, Mitogen-activated protein kinase (MAPK): Janus kinase/signal transducer and activator of transcription (JAK/STAT): Reactive oxygen species-mediated pathways, Cytokine signalling cascades, Single-drug therapy often targets only one pathway, whereas dual drug-loaded systems can inhibit multiple inflammatory mediators concurrently.[33] For example, NSAIDs combined with corticosteroids provide enhanced suppression of prostaglandins and inflammatory cytokines., Antioxidants co-delivered with anti-inflammatory drugs reduce

oxidative stress while suppressing inflammatory signaling[34], Natural phytochemicals combined with synthetic drugs may enhance efficacy while minimizing toxicity, Gene therapeutics combined with anti-inflammatory agents may regulate inflammatory gene expression and immune responses simultaneously. Liposomal co-delivery systems also facilitate synchronized pharmacokinetics of both drugs, ensuring simultaneous arrival at target tissues and coordinated therapeutic action. Controlled release from liposomal carriers maintains optimal drug ratios at inflamed sites, which is critical for achieving synergistic interactions.[35] Additionally, liposomal accumulation at inflamed tissues occurs through enhanced vascular permeability and inflammatory microenvironment targeting. Macrophages and activated immune cells readily internalize liposomes, enabling intracellular drug delivery and improved modulation of inflammatory signaling pathways.[36] Recent studies have demonstrated that dual drug-loaded liposomes significantly reduce inflammatory cytokine production, oxidative stress, immune cell infiltration, and tissue damage compared to free drug combinations. These findings highlight the immense potential of liposomal co-delivery systems as advanced therapeutic platforms for the treatment of chronic inflammatory diseases.[37]

3. Formulation Strategies and Characterization

The successful development of dual drug-loaded liposomal systems for anti-inflammatory applications requires careful optimization of formulation parameters, encapsulation techniques, lipid composition, and physicochemical properties.[38] The co-delivery of two therapeutic agents within a single liposomal carrier presents unique formulation challenges due to differences in drug solubility, stability, molecular weight, and physicochemical compatibility. Appropriate formulation strategies are therefore essential to achieve high encapsulation efficiency, controlled drug release, prolonged stability, and enhanced therapeutic performance.[39]

In recent years, significant advancements in liposomal engineering have enabled the development of multifunctional and targeted dual drug-loaded systems capable of improving anti-inflammatory efficacy while minimizing systemic toxicity. Comprehensive physicochemical characterization is critical for evaluating formulation quality, stability, and biological performance.[40]

3.1 Methods for Dual Drug Encapsulation

Dual drug encapsulation within liposomal systems can be achieved through various formulation techniques depending on the physicochemical properties of the therapeutic agents and desired release characteristics. The choice of preparation method significantly influences particle size, drug loading efficiency, bilayer integrity, and stability.[41]

3.1.1 Thin-Film Hydration Metho.

The thin-film hydration method is one of the most commonly employed techniques for preparing dual drug-loaded liposomes. In this method, phospholipids, cholesterol, and lipophilic drugs are dissolved in organic solvents such as chloroform or methanol. The solvent is subsequently removed under reduced pressure using a rotary evaporator, forming a thin lipid film on the flask surface.[42]

The lipid film is then hydrated with an aqueous phase containing hydrophilic drugs under controlled temperature and agitation conditions, resulting in spontaneous formation of multilamellar vesicles. Further size reduction may be achieved through sonication, extrusion, or homogenization.[43]

This method is widely used due to its simplicity, versatility, and ability to encapsulate both hydrophilic and lipophilic therapeutic agents simultaneously.

3.1.2 Reverse-Phase Evaporation Method

In this technique, lipids are dissolved in an organic phase and emulsified with an aqueous drug solution to form a water-in-oil emulsion. Solvent evaporation leads to liposome formation with high encapsulation efficiency, particularly for hydrophilic compounds.[15]

The reverse-phase evaporation method is advantageous for co-encapsulation of drugs with different solubility characteristics and provides larger aqueous core volumes for enhanced drug loading.[44]

3.1.3 Ethanol Injection Method

The ethanol injection method involves rapid injection of lipid solutions dissolved in ethanol into an aqueous phase containing hydrophilic drugs. Spontaneous vesicle formation occurs upon solvent diffusion.[45]

This method is simple, scalable, and suitable for thermosensitive compounds but may produce relatively heterogeneous particle size distributions.

3.1.4 Microfluidization and High-Pressure Homogenization

Advanced techniques such as microfluidization and high-pressure homogenization enable controlled production of nanosized liposomes with narrow size distributions and improved reproducibility. These methods are particularly useful for large-scale manufacturing and industrial applications.[46]

3.1.5 Active Loading Techniques

Active or remote loading techniques utilize transmembrane ion or pH gradients to improve encapsulation efficiency of weakly acidic or basic drugs. These methods enhance drug retention and provide controlled release properties.[47]

Remote loading is especially advantageous for achieving high drug loading efficiencies and stable co-encapsulation in dual drug-loaded systems.

3.2 Selection of Lipids and Bioactive Compounds

The therapeutic performance and stability of liposomal formulations are strongly influenced by the selection of lipids, surfactants, and bioactive compounds.[38]

3.2.1 Selection of Lipids

Phospholipids form the structural framework of liposomes and determine membrane fluidity, permeability, stability, and drug release characteristics.[48] Commonly used phospholipids include, Phosphatidylcholine, Dipalmitoyl phosphatidylcholine (DPPC): Hydrogenated soy phosphatidylcholine (HSPC): Phosphatidylethanolamine, Sphingomyelin Cholesterol is frequently incorporated to improve membrane rigidity and reduce permeability, thereby enhancing liposomal stability and minimizing premature drug leakage.[49] The choice of lipid composition depends on factors such as, Drug compatibility, Desired release profile, Surface charge, Bilayer fluidity, Target tissue, Stability requirements.

3.2.2 Selection of Bioactive Compounds

Dual drug-loaded liposomes may contain combinations of therapeutic agents with complementary or synergistic anti-inflammatory mechanisms.[38] Common combinations include, NSAIDs + corticosteroids, Anti-inflammatory drugs + antioxidants, Synthetic drugs + phytochemicals, Immunomodulators + biologics, Small molecules + nucleic acids

Examples of frequently investigated bioactive compounds include, Curcumin, Dexamethasone, Celecoxib, Diclofenac, Resveratrol, Quercetin, Methotrexate, Ibuprofen, The physicochemical properties of drugs, including solubility, pKa, lipophilicity, and molecular size, significantly affect encapsulation efficiency and localization within liposomal compartments.[50]

3.3 Physicochemical Characterization

Comprehensive physicochemical characterization is essential to evaluate the quality, performance, and stability of dual drug-loaded liposomal systems.[51]

3.3.1 Particle Size and Zeta Potential

Particle size is one of the most critical parameters influencing biodistribution, cellular uptake, circulation time, and accumulation at inflamed tissues. Smaller liposomes generally exhibit, enhanced cellular internalization, Improved tissue penetration, Increased stability, better inflammatory site targeting Dynamic light scattering (DLS) is commonly employed to determine particle size and polydispersity index (PDI). Zeta potential measures surface charge and provides information regarding colloidal stability. High absolute zeta potential values generate electrostatic repulsion between vesicles, preventing aggregation and improving formulation stability.[52] Surface charge also influences interactions with biological membranes and immune cells.

3.3.2 Entrapment Efficiency

Entrapment efficiency represents the percentage of therapeutic agents successfully encapsulated within liposomes relative to the total amount added during formulation.[53] High entrapment efficiency is desirable for, sustained therapeutic action, Reduced drug wastage, Improved pharmacokinetics, enhanced therapeutic efficacy, Entrapment efficiency depends on multiple factors including, Lipid composition, Drug-lipid compatibility, Preparation method, Drug concentration, Surface charge, Bilayer fluidity, Methods such as ultracentrifugation, dialysis, and gel filtration chromatography are commonly used to separate free drug from encapsulated drug prior to analysis.

3.3.3 Drug Release Behavior

Drug release behavior significantly influences therapeutic performance and dosing frequency. Dual drug-loaded liposomal systems are generally designed to provide Sustained release, Controlled release, Sequential release, Stimuli-responsive release enhanced studies are

commonly performed using dialysis membrane techniques or diffusion systems under physiological conditions.[54] Drug release kinetics may follow, Diffusion-controlled mechanisms. Bilayer erosion, Liposomal destabilization, Stimuli-triggered release, Controlled release maintains therapeutic drug concentrations at inflamed sites while minimizing systemic exposure and adverse effects. Stimuli-responsive liposomes may release drugs selectively in response to, Acidic pH, Oxidative stress, Enzymatic activity, Temperature changes, these conditions are often associated with inflammatory microenvironments.[55]

3.4 Stability Considerations

Stability is a major challenge in the development of liposomal formulations due to their complex structural organization and susceptibility to physicochemical degradation.[56] Several instability phenomena may occur during storage, including, Vesicle aggregation, Fusion, Drug leakage, Lipid oxidation, Hydrolysis, Phase separation.

3.4.1 Physical Stability

Physical stability involves maintaining vesicle size, morphology, and dispersion characteristics over time. Factors affecting physical stability include, Lipid composition, Surface charge, Temperature, Storage conditions, Presence of stabilizers, PEGylation and cholesterol incorporation are commonly employed to enhance membrane stability and prolong shelf life.[57]

3.4.2 Chemical Stability

Phospholipids are susceptible to oxidative degradation and hydrolysis, particularly under elevated temperature, light exposure, and oxygen-rich environments. Antioxidants such as vitamin E and butylated hydroxytoluene (BHT) may be incorporated to reduce lipid oxidation.[58] Lyophilization (freeze-drying) is another important strategy used to improve long-term stability of liposomal formulations.

3.4.3 Biological Stability

Biological stability refers to the ability of liposomes to maintain structural integrity in biological fluids without rapid clearance by the immune system.[59]

Surface modification with polyethylene glycol (PEG) reduces opsonization and uptake by macrophages, thereby prolonging circulation time.

3.4.4 Storage Stability

Long-term storage stability studies are essential to evaluate changes in, Particle size, Zeta potential, Drug content, Entrapment efficiency, Release profile, Visual appearance, these studies are typically conducted according to International Council for Harmonization (ICH) guidelines. Overall, appropriate formulation strategies and comprehensive physicochemical characterization are essential for the successful development of stable and effective dual drug-loaded liposomal systems. Advances in lipid engineering, encapsulation techniques, and targeted delivery approaches continue to enhance the therapeutic potential of liposomal nanocarriers for anti-inflammatory applications.[60]

4. Therapeutic Applications of Dual Drug-Loaded Liposomes in Inflammation

Dual drug-loaded liposomal systems have emerged as highly promising therapeutic platforms for the treatment of inflammatory disorders due to their ability to simultaneously deliver multiple bioactive agents with complementary mechanisms of action. These advanced nanocarriers improve therapeutic efficacy by enhancing drug bioavailability, reducing systemic toxicity, prolonging circulation time, and enabling targeted delivery to inflamed tissues. The co-encapsulation of synthetic anti-inflammatory drugs with natural bioactive compounds, antioxidants, or immunomodulators has demonstrated significant synergistic effects in both experimental and preclinical models of inflammation.[34]

Inflammatory diseases are characterized by complex and interconnected signaling pathways involving cytokines, oxidative stress, immune cell activation, and inflammatory enzymes. Single-drug therapies often fail to sufficiently regulate these multifactorial processes. In contrast, dual drug-loaded liposomal systems can simultaneously target multiple inflammatory mediators and signaling pathways, thereby improving therapeutic outcomes and minimizing adverse effects.[61]

Table 1: Summary of dual drug-loaded liposomal formulations developed for anti-inflammatory applications, including encapsulated agents, formulation characteristics, and therapeutic outcomes.

Drugs/Bioactives	Lipid Composition	Particle Size (nm)	Entrapment Efficiency (%)	Release Profile	Anti-Inflammatory Activity
Curcumin + Dexamethasone	Phosphatidylcholine + Cholesterol + DSPE-PEG	120–165	82–91	Sustained release over 48 h	Significant reduction of TNF-α, IL-6, and NF-κB activation in macrophages
Resveratrol + Celecoxib	HSPC + Cholesterol	140–190	80–89	Biphasic controlled release	Synergistic inhibition of COX-2 and oxidative stress in arthritic models
Quercetin + Methotrexate	DPPC + Cholesterol + PEGylated lipids	110–170	78–92	Prolonged release profile	Reduced synovial inflammation and cartilage destruction in rheumatoid arthritis
Ibuprofen + Curcumin	Soy phosphatidylcholine + Cholesterol	150–210	75–88	Sustained diffusion-controlled release	Enhanced suppression of inflammatory cytokines and ROS generation
Dexamethasone + Berberine	HSPC + Cholesterol + DSPE-PEG2000	125–180	84–93	Controlled release for 72 h	Improved anti-inflammatory efficacy and reduced pulmonary inflammation
Prednisolone + Resveratrol	Egg phosphatidylcholine + Cholesterol	130–195	79–90	Sequential and sustained release	Decreased oxidative stress and inflammatory mediator production
Curcumin + Diclofenac	DPPC + Cholesterol + Stearyl amine	100–160	81–95	Controlled release behavior	Enhanced inhibition of prostaglandin synthesis and edema formation
Celecoxib + Quercetin	Phosphatidylcholine + Cholesterol + PEG-lipid	145–220	76–89	Sustained release up to 48 h	Improved suppression of MAPK and NF-κB signaling pathways
Methotrexate + Curcumin	HSPC + Cholesterol + PEGylated phospholipid	115–175	83–94	Long-term sustained release	Significant reduction in joint inflammation and immune cell infiltration
Dexamethasone + EGCG	DSPC + Cholesterol + PEG2000	135–200	80–90	Controlled and pH-responsive release	Enhanced anti-inflammatory and antioxidant activity in colitis models

4.1 Liposomal Co-Delivery of Natural Compounds and Synthetic Drugs

The combination of natural phytochemicals with conventional synthetic anti-inflammatory drugs within liposomal systems has gained considerable attention in recent years. Natural compounds possess potent antioxidant, anti-inflammatory, and immunomodulatory

properties, while synthetic drugs provide rapid and strong pharmacological effects. Co-delivery of these agents within a single liposomal carrier enhances therapeutic synergy and may reduce the required dose of synthetic drugs, thereby minimizing toxicity.[62] Several natural bioactive compounds have been extensively investigated in liposomal co-delivery systems, including, Curcumin, Resveratrol, Quercetin, Epigallocatechin gallate (EGCG): Berberine, Boswellic acid, these phytochemicals exhibit anti-inflammatory activity through inhibition of oxidative stress, suppression of inflammatory cytokines, and modulation of signaling pathways such as NF-κB and MAPK.[63] Synthetic anti-inflammatory drugs commonly incorporated into dual drug-loaded liposomes include, Dexamethasone, Celecoxib, Diclofenac, Ibuprofen, Methotrexate, Prednisolone, for example, liposomal co-delivery of curcumin and dexamethasone has demonstrated enhanced suppression of inflammatory cytokines and oxidative stress compared to individual drug formulations. Similarly, co-encapsulation of resveratrol with celecoxib has shown synergistic inhibition of cyclooxygenase-2 (COX-2) and reactive oxygen species production in inflammatory disease models.[64] The liposomal encapsulation of natural compounds also improves their poor aqueous solubility, low bioavailability, and rapid metabolism, thereby enhancing therapeutic effectiveness.[65]

4.2 Modulation of Inflammatory Mediators and Signaling Pathways

The anti-inflammatory efficacy of dual drug-loaded liposomal systems is primarily associated with their ability to regulate multiple inflammatory mediators and intracellular signaling pathways simultaneously.[66] Inflammatory responses involve the activation of several molecular pathways, including, nuclear factor-kappa B (NF-κB): Cyclooxygenase (COX) pathway, Lipoxygenase (LOX) pathway, Mitogen-activated protein kinase (MAPK) pathway, Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, Reactive oxygen species (ROS)-mediated signaling, Dual drug-loaded liposomes can effectively suppress these pathways through synergistic mechanisms.[61]

4.2.1 Inhibition of Pro-Inflammatory Cytokines

Liposome-mediated co-delivery systems have demonstrated significant inhibition of pro-inflammatory cytokines such as, Tumor necrosis factor-alpha (TNF-α): Interleukin-1β (IL-1β): Interleukin-6 (IL-6): Interferon-gamma (IFN-γ): Reduction of these cytokines decreases inflammatory cell recruitment, vascular permeability, tissue damage, and chronic inflammatory progression.[67]

4.2.2 Suppression of NF-κB Signaling

NF-κB is a key transcription factor regulating the expression of inflammatory mediators including COX-2, inducible nitric oxide synthase (iNOS): and cytokines. Dual drug-loaded liposomes significantly inhibit NF-κB activation by suppressing IκB phosphorylation and preventing nuclear translocation of NF-Kb.[68] Natural compounds such as curcumin and quercetin exhibit strong NF-κB inhibitory activity, while corticosteroids and NSAIDs provide additional anti-inflammatory effects.

4.2.3 Regulation of Oxidative Stress

Oxidative stress plays a critical role in inflammatory tissue injury and disease progression. Liposomal co-delivery of antioxidants with anti-inflammatory drugs reduces intracellular ROS generation and enhances endogenous antioxidant defense systems.[69] These systems increase the activity of antioxidant enzymes such as, Superoxide dismutase (SOD): Catalase, Glutathione peroxidase, Reduction of oxidative stress contributes significantly to attenuation of inflammatory responses.

4.2.4 Modulation of COX-2 and iNOS Expression

Dual drug-loaded liposomes effectively suppress expression of inflammatory enzymes including COX-2 and iNOS, thereby reducing prostaglandin and nitric oxide production. The simultaneous targeting of multiple inflammatory mediators provides broader therapeutic coverage and improved efficacy compared to monotherapy.[70]

4.3 In Vitro and In Vivo Anti-Inflammatory Studies

Extensive in vitro and in vivo investigations have validated the therapeutic potential of dual drug-loaded liposomal systems in inflammatory disease models.

4.3.1 In Vitro Studies

Cell culture studies using macrophages, epithelial cells, fibroblasts, and immune cells have demonstrated that dual drug-loaded liposomes significantly reduce inflammatory mediator production and improve cellular uptake compared to free drugs.[60] Commonly employed experimental models include, Lipopolysaccharide (LPS)-stimulated macrophages, Cytokine-induced inflammatory cell models, Oxidative stress-induced cellular inflammation These studies have shown, Reduced cytokine secretion, Inhibition of inflammatory gene expression, Decreased ROS production, Enhanced intracellular drug accumulation, Improved

cytocompatibility, Liposomal formulations also exhibit controlled drug release and prolonged anti-inflammatory activity at the cellular level.[71]

4.3.2 In Vivo Studies

Animal models of inflammation have further confirmed the therapeutic superiority of dual drug-loaded liposomes. Common experimental models include, Carrageenan-induced paw edema, Rheumatoid arthritis models, Colitis models, Psoriasis models, Pulmonary inflammation models, Neuroinflammation models, Dual drug-loaded liposomes have demonstrated, Reduced inflammatory edema, Decreased immune cell infiltration, Suppression of cytokine production, Reduced tissue damage, Improved histopathological outcomes, Enhanced survival rates For example, liposomal co-delivery of methotrexate and curcumin in rheumatoid arthritis models significantly reduced synovial inflammation and cartilage destruction compared to conventional therapy.[72] Similarly, dual liposomal formulations containing dexamethasone and antioxidants have shown superior efficacy in pulmonary inflammatory disorders by reducing oxidative stress and inflammatory cytokine production.[73]

4.4 Targeted and Controlled Drug Delivery Approaches

One of the major advantages of dual drug-loaded liposomal systems is their ability to achieve targeted and controlled drug delivery to inflamed tissues.[25]

4.4.1 Passive Targeting

Inflamed tissues often exhibit enhanced vascular permeability due to endothelial dysfunction and inflammatory mediator-induced vasodilation. Liposomes can accumulate passively at these sites through enhanced permeability and retention (EPR)-like mechanisms associated with inflammation.[21] Nanometer-sized liposomes preferentially extravasate into inflamed tissues and remain localized due to impaired lymphatic drainage.

4.4.2 Active Targeting

Active targeting strategies involve surface modification of liposomes with ligands capable of binding specific receptors overexpressed on inflammatory cells or tissues. Common targeting ligands include, Antibodies, Peptides, Folate, Hyaluronic acid, Mannose, Transferrin, these modifications enhance selective uptake by macrophages, neutrophils, dendritic cells, and activated endothelial cells, thereby improving site-specific delivery and reducing systemic toxicity.[74]

4.4.3 Stimuli-Responsive Drug Release

Stimuli-responsive liposomes are designed to release encapsulated drugs selectively in response to pathological conditions associated with inflammation, these triggers include, Acidic pH, Reactive oxygen species, Enzymatic activity, Elevated temperature, Inflamed tissues frequently exhibit acidic microenvironments and oxidative stress, making them suitable targets for responsive drug release systems.[75]

4.4.4 Controlled Release Systems

Controlled release liposomal formulations provide sustained delivery of anti-inflammatory agents over extended periods, maintaining therapeutic concentrations while minimizing repeated dosing.[38] This controlled release behavior, improves therapeutic efficacy, reduces systemic exposure, minimizes adverse effects, enhances patient compliance, Recent advances in lipid engineering, nanotechnology, and surface functionalization have further improved the targeting efficiency and therapeutic performance of dual drug-loaded liposomal systems.[38] Overall, dual drug-loaded liposomes represent highly versatile and effective nanotherapeutic platforms for inflammatory disease management. Their ability to co-deliver synergistic therapeutic agents, regulate multiple inflammatory pathways, and achieve targeted controlled release highlights their significant potential for future clinical applications in chronic inflammatory disorders.[25]

5. Challenges, Future Perspectives, and Conclusion

5.1 Scale-Up and Formulation Challenges

Despite the remarkable therapeutic potential of dual drug-loaded liposomal systems for anti-inflammatory applications, several formulation and manufacturing challenges continue to limit their successful translation from laboratory research to clinical and commercial use. The complexity associated with simultaneous encapsulation of multiple therapeutic agents, maintenance of liposomal stability, and large-scale production presents significant scientific and technological obstacles.[30] One of the primary formulation challenges involves achieving efficient co-encapsulation of two drugs with distinct physicochemical properties. Differences in aqueous solubility, lipophilicity, molecular size, ionization behavior, and chemical stability may influence drug loading efficiency and localization within liposomal compartments. Hydrophilic drugs are generally entrapped within the aqueous core, whereas lipophilic drugs partition into the phospholipid bilayer, making simultaneous optimization difficult.[76] Maintaining the optimal ratio of co-encapsulated drugs is another important concern, as

therapeutic synergy often depends on precise drug proportions. Premature leakage or differential release rates may alter the intended therapeutic ratio and compromise efficacy.[77] Liposomal instability during storage also remains a major limitation. Liposomes are susceptible to several degradation processes, including, Vesicle aggregation, Fusion, Phospholipid oxidation, Hydrolysis, Drug leakage, Phase separation, these instability phenomena can significantly affect particle size, drug release behavior, encapsulation efficiency, and therapeutic performance.[78] Scale-up of liposomal formulations presents additional technical difficulties. Laboratory-scale preparation methods such as thin-film hydration and sonication often lack reproducibility and scalability for industrial manufacturing. Producing liposomes with consistent particle size distribution, surface characteristics, and encapsulation efficiency on a commercial scale remains challenging.[79] Furthermore, large-scale manufacturing requires strict control over, Process parameters, Sterility, Batch-to-batch consistency, Quality assurance, Residual solvent levels, Advanced manufacturing techniques such as microfluidics, high-pressure homogenization, and continuous flow systems are being explored to improve scalability and reproducibility., Another challenge involves the high production cost associated with specialized phospholipids, targeting ligands, surface modifiers, and sophisticated manufacturing equipment. These factors may limit widespread commercial accessibility of advanced liposomal therapeutics.[30]

5.2 Regulatory and Clinical Considerations

The clinical translation and regulatory approval of liposomal nanomedicines remain highly complex due to the unique physicochemical and biological properties of nanoparticle-based drug delivery systems.[30] Regulatory agencies require extensive characterization of liposomal formulations, including, Particle size distribution, Surface charge, Encapsulation efficiency, Drug release kinetics, Stability, Sterility, Biodistribution, Toxicological profile, However, standardized regulatory guidelines specifically designed for multifunctional nanocarrier systems are still evolving. Conventional pharmaceutical evaluation methods may not adequately address the complexity of dual drug-loaded liposomal formulations.[25] Safety evaluation is particularly important because nanoparticle interactions with biological systems may differ significantly from conventional dosage forms. Although liposomes are generally considered biocompatible and biodegradable, long-term safety concerns remain regarding, Immunogenicity, complement activation, Accumulation in organs, altered biodistribution, unexpected toxicity, PEGylated liposomes, for example, may induce accelerated blood

clearance phenomena or hypersensitivity reactions in some patients following repeated administration.[80] Clinical translation also faces challenges associated with variability in patient physiology, disease progression, immune response, and inflammatory microenvironment characteristics. These factors may influence liposomal distribution, cellular uptake, and therapeutic efficacy.[30] Large-scale clinical trials evaluating dual drug-loaded liposomal systems for inflammatory diseases are still relatively limited. Most current evidence is derived from in vitro studies and animal models. Comprehensive clinical studies are required to establish, Therapeutic efficacy, Long-term safety, Pharmacokinetic behavior, Optimal dosing regimens, Comparative effectiveness, Patient compliance, Additionally, regulatory approval processes for combination nanomedicines may be more complicated because multiple active pharmaceutical ingredients must be evaluated simultaneously for efficacy, interactions, and toxicity.[81]

5.3 Emerging Trends in Multifunctional Liposomal Systems

Rapid advances in nanotechnology, biomaterials engineering, molecular biology, and precision medicine are driving the development of next-generation multifunctional liposomal systems for anti-inflammatory therapy.[38] One major emerging trend involves the development of stimuli-responsive liposomes capable of site-specific drug release in response to pathological conditions associated with inflammation. These smart liposomes respond to triggers such as: Acidic pH, Reactive oxygen species (ROS): Enzymatic activity, Temperature changes, Ultrasound, Magnetic fields, Inflamed tissues frequently exhibit acidic and oxidative microenvironments, enabling selective drug release at diseased sites while minimizing systemic exposure.[82] Targeted liposomal systems are also gaining considerable attention. Surface-functionalized liposomes containing antibodies, peptides, aptamers, folate, mannose, or hyaluronic acid can selectively target inflammatory cells and tissues, thereby enhancing therapeutic precision.[83] Another important advancement is the incorporation of gene therapeutics and nucleic acid-based medicines into liposomal systems. Co-delivery of small interfering RNA (siRNA): microRNA, plasmid DNA, or CRISPR-associated therapeutics with anti-inflammatory drugs offers opportunities for simultaneous modulation of inflammatory gene expression and signaling pathways.[11] Theranostic liposomes combining diagnostic and therapeutic functions represent another promising research direction. These multifunctional systems integrate imaging agents with therapeutic compounds to enable real-time monitoring of drug distribution, disease progression, and treatment response.[25] Combination strategies involving natural bioactive compounds and

synthetic drugs are increasingly being explored to enhance synergistic anti-inflammatory effects while reducing toxicity. Phytochemicals such as curcumin, quercetin, resveratrol, and berberine have shown significant promise when co-delivered with conventional anti-inflammatory agents.[84] Artificial intelligence (AI) and computational modeling are also emerging as valuable tools for liposomal formulation optimization, prediction of nanoparticle behavior, and personalized nanomedicine development. Machine learning approaches may accelerate the design of highly efficient liposomal systems with improved targeting and therapeutic performance.[85] Other future trends include: Hybrid lipid-polymer nanocarriers, Biomimetic liposomes, Exosome-inspired vesicles, Nanogel-integrated liposomes, Personalized nanotherapeutics, Transdermal and mucosal liposomal delivery systems These innovations are expected to significantly improve the clinical applicability and therapeutic effectiveness of anti-inflammatory nanomedicines.[11]

5.4 Conclusion and Future Outlook

Dual drug-loaded liposomal systems represent highly advanced and versatile nanotherapeutic platforms for the treatment of inflammatory disorders. Their unique ability to co-deliver multiple therapeutic agents within a single carrier enables simultaneous modulation of diverse inflammatory pathways, resulting in enhanced therapeutic efficacy and reduced systemic toxicity.[20] Liposomal nanocarriers offer several advantages over conventional anti-inflammatory therapies, including improved drug solubility, enhanced bioavailability, prolonged circulation time, targeted tissue accumulation, controlled drug release, and better patient compliance. The co-encapsulation of synthetic drugs with natural bioactive compounds or gene therapeutics further enhances synergistic therapeutic effects and broadens the scope of anti-inflammatory treatment strategies.[34] Extensive in vitro and in vivo studies have demonstrated the significant potential of dual drug-loaded liposomes in the management of chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, psoriasis, pulmonary inflammation, and neuroinflammatory disorders. Advances in lipid engineering, targeting strategies, and stimuli-responsive systems continue to improve the efficiency and precision of liposomal drug delivery.[38] Nevertheless, important challenges related to formulation stability, large-scale manufacturing, regulatory approval, and long-term clinical safety must still be addressed before widespread clinical translation can be achieved. Standardization of manufacturing protocols, improved characterization methods, and comprehensive clinical evaluation will be essential for successful commercialization.[86] Future research should focus on the development of multifunctional, targeted, and

personalized liposomal systems capable of responding to disease-specific microenvironments. Integration of nanotechnology with molecular therapeutics, artificial intelligence, and precision medicine approaches is expected to revolutionize the field of anti-inflammatory drug delivery. Overall, dual drug-loaded liposomal systems hold tremendous promise as next-generation therapeutic platforms for inflammatory disease management. Continued interdisciplinary research and technological advancements are likely to facilitate the development of safer, more effective, and clinically translatable liposomal nanomedicines in the near future.[87]

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