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A LIPOSOMES AS A NOVEL DRUG DELIVERY SYSTEM: FORMULATION, CHARACTERIZATION, AND THERAPEUTIC APPLICATIONS

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ABSTRACT

Liposomes are among the most extensively investigated and clinically validated nanocarrier-based drug delivery systems, owing to their unique structural characteristics, biocompatibility, and ability to encapsulate a wide variety of therapeutic agents. Structurally, liposomes are spherical vesicles composed of one or more phospholipid bilayers enclosing an aqueous core, enabling the simultaneous delivery of both hydrophilic and lipophilic drugs. This comprehensive review highlights the formulation strategies, characterization techniques, and therapeutic applications of liposomal drug delivery systems. Various methods of liposome preparation, including thin-film hydration, reverse-phase evaporation, ethanol injection, and micro fluidization, are discussed in detail along with factors affecting liposome size, stability, and drug loading efficiency. The review further elaborates on critical characterization parameters such as vesicle size, zeta potential, encapsulation efficiency, in vitro release behaviour, and stability studies. Therapeutic applications of liposomes in cancer therapy, infectious diseases, vaccine delivery, gene therapy, and targeted drug delivery are critically examined, with emphasis on clinically approved liposomal products. Despite their advantages, liposomal systems face challenges related to physical instability, rapid clearance, and large-scale manufacturing. Emerging advancements such as PEGylated liposomes,

ligand-targeted liposomes, and stimuli-responsive liposomes are paving the way for next-generation personalized therapeutics. Overall, liposomes continue to represent a versatile and promising platform in modern drug delivery science.

KEYWORDS: Liposomes, Novel drug delivery system, Nanocarriers, Targeted drug delivery, Encapsulation efficiency, PEGylation, Therapeutic applications.

INTRODUCTION

Novel Drug Delivery Systems (NDDS) have emerged as a critical area of pharmaceutical research aimed at overcoming the limitations associated with conventional dosage forms, such as poor bioavailability, lack of site specificity, systemic toxicity, and frequent dosing requirements. Among the various nanocarrier-based delivery platforms, liposomes have gained exceptional recognition due to their structural similarity to biological membranes, high biocompatibility, and clinical versatility.[1]

Liposomes were first described by Alec D. Bangham in 1965 while studying phospholipid dispersions, and since then they have evolved into one of the most extensively researched drug delivery systems. Structurally, liposomes are spherical vesicles composed of one or more concentric phospholipid bilayers enclosing an aqueous core. This unique amphiphilic architecture enables the encapsulation of both hydrophilic drugs (within the aqueous core) and lipophilic drugs (within the lipid bilayer), making liposomes highly adaptable carriers for a broad range of therapeutic agents.[2]

One of the most significant advantages of liposomal drug delivery is its ability to alter the pharmacokinetic and biodistribution profiles of encapsulated drugs. Liposomes can protect drugs from enzymatic degradation, reduce rapid systemic clearance, and prolong circulation time, thereby enhancing therapeutic efficacy while minimizing dose-related toxicity. These features are particularly beneficial for drugs with narrow therapeutic indices, poor aqueous solubility, or high systemic toxicity, such as anticancer and antifungal agents.[3]

Conventional chemotherapy and antimicrobial therapies are often associated with severe adverse effects due to nonspecific distribution of drugs to healthy tissues. Liposomal encapsulation significantly reduces off-target toxicity by preferential drug accumulation at diseased sites through passive targeting mechanisms such as the enhanced permeability and retention (EPR) effect, as well as active targeting strategies using ligands or antibodies. As a

result, liposomal formulations have demonstrated improved safety profiles and patient tolerability in clinical settings.[4,5]

Advances in lipid chemistry, nanotechnology, and surface engineering have led to the development of next-generation liposomes, including PEGylated (stealth) liposomes, immunoliposomes, stimuli-responsive liposomes, and theranostic liposomes. These sophisticated systems are capable of prolonged circulation, site-specific targeting, controlled drug release, and real-time imaging. Consequently, liposomes have become a cornerstone of modern NDDS and continue to play a pivotal role in personalized and precision medicine.[6]

ADVANTAGES OF LIPOSOMAL DRUG DELIVERY SYSTEMS

1. Biocompatibility and Biodegradability

Liposomes are made from phospholipids that are similar to the lipids present in human cell membranes. Because of this similarity, the body easily accepts liposomes without triggering significant immune or toxic reactions. After delivering the drug, liposomes are naturally broken down into fatty acids and glycerol, which are safely metabolized or eliminated from the body. This makes liposomes non-toxic, safe, and suitable for repeated or long-term administration.

2. Versatile Drug Encapsulation

The unique bilayer structure of liposomes allows them to carry both hydrophilic and lipophilic drugs simultaneously. Hydrophilic drugs are entrapped inside the aqueous core, while lipophilic drugs are incorporated within the lipid bilayer. This dual-encapsulation capability makes liposomes highly versatile carriers for a wide range of therapeutic agents, including anticancer drugs, antibiotics, vaccines, and genes.[7]

3. Improved Bioavailability

Many drugs suffer from poor solubility and limited absorption when administered conventionally. Liposomes enhance drug solubility and protect drugs from degradation, leading to increased absorption and prolonged circulation time. As a result, a higher fraction of the administered drug reaches systemic circulation, significantly improving bioavailability, especially for poorly water-soluble and unstable drugs.[8]

4. Targeted Drug Delivery

Liposomes can be designed to deliver drugs specifically to diseased tissues. Passive targeting occurs through the enhanced permeability and retention (EPR) effect, where liposomes accumulate in tumor tissues due to leaky vasculature. Active targeting is achieved by attaching ligands, antibodies, or peptides to the liposome surface, allowing selective binding to target cells. This improves therapeutic efficiency while minimizing side effects.[9]

5. Reduced Drug Toxicity

By encapsulating drugs within liposomes, direct exposure of healthy tissues to toxic drugs is significantly reduced. This is especially beneficial in anticancer therapy, where potent cytotoxic drugs can damage normal cells. Liposomal formulations concentrate the drug at the target site, thereby reducing systemic toxicity and improving patient tolerability.[10]

6. Controlled and Sustained Release

Liposomes can be engineered to release drugs slowly and in a controlled manner over an extended period. The lipid bilayer acts avoiding sudden release of the drug. This helps maintain constant therapeutic drug levels, reduces dosing frequency, and prevents fluctuations in plasma drug concentration, leading to better therapeutic outcomes.[11]

7. Protection from Degradation

Encapsulation within liposomes protects drugs from chemical degradation and enzymatic breakdown in the bloodstream or gastrointestinal tract. This is particularly important for sensitive molecules such as proteins, peptides, nucleic acids, and vaccines, which are easily degraded under physiological conditions.[12]

8. Clinical Acceptance

Liposomes have gained widespread clinical acceptance due to their proven safety and efficacy. Several liposomal formulations, such as Doxil® (doxorubicin) and AmBisome® (amphotericin B), have been approved by regulatory authorities like the US FDA. Their successful commercialization confirms the reliability of liposomes as an effective drug delivery system.

LIMITATIONS OF LIPOSOMAL DRUG DELIVERY SYSTEMS

1. Physical and Chemical Instability

Liposomes are inherently unstable systems. During storage or circulation, liposomal vesicles may undergo aggregation (clumping together) or fusion (merging of vesicles), which alters their size and drug release profile. Additionally, phospholipids—especially unsaturated ones—are prone to oxidation and hydrolysis, leading to degradation of the lipid bilayer and loss of drug content.[13]

2. Short Shelf Life: Due to their susceptibility to oxidation, hydrolysis, and physical instability, liposomes often have a limited shelf life. They usually require refrigerated storage, protection from light, and inert atmosphere packaging. These stringent storage conditions limit their convenience and increase the overall cost of distribution and handling.[14]

3. High Manufacturing Cost

The preparation of liposomes involves expensive raw materials (high-purity phospholipids and cholesterol), specialized equipment, and complex processing steps. Additionally, advanced techniques such as PEGylation, sterilization, and particle size control further increase production costs, making liposomal formulations more expensive than conventional dosage forms.[15]

4. Rapid Clearance by the Reticuloendothelial System (RES)

Conventional liposomes are quickly recognized as foreign particles by the reticuloendothelial system, particularly macrophages in the liver and spleen. This leads to rapid uptake and clearance from systemic circulation, reducing the time available for the liposomes to reach the target site and lowering therapeutic efficacy.

5. Drug Leakage

Some drugs may leak out of liposomes during storage or after administration due to bilayer permeability, lipid phase transition, or weak drug–lipid interactions. Premature drug leakage can result in reduced dose delivery to the target site and increased systemic side effects.

6. Limited Drug Loading

Liposomes have limited capacity for drug encapsulation, especially for highly water-soluble drugs, which may easily diffuse out of the aqueous core. This limitation often requires

administration of larger volumes or higher lipid content to achieve the desired therapeutic dose.

7. Scale-up Challenges

Scaling up liposome production from laboratory to industrial level is challenging due to difficulties in maintaining consistent vesicle size, lamellarity, drug loading, and stability. Variations between batches can affect product quality, safety, and therapeutic performance, posing significant challenges for commercial manufacturing.[16]

STRUCTURE AND COMPOSITION OF LIPOSOMES

Liposomes are spherical vesicular systems composed mainly of amphiphilic phospholipids arranged in one or more concentric bilayers surrounding an aqueous core. When phospholipids are dispersed in an aqueous medium, they spontaneously self-assemble due to their amphiphilic nature, leading to the formation of closed bilayer structures known as liposomes.

1. Phospholipid Components: Each phospholipid molecule consists of two distinct regions.

- **Hydrophilic (polar) head group:** This portion is water-loving and usually contains phosphate groups linked to choline, ethanolamine, serine, or inositol. The hydrophilic heads are oriented towards the aqueous environment, both on the inner and outer surfaces of the liposome.
- **Hydrophobic (non-polar) fatty acid tails**: These long hydrocarbon chains are water-repelling and face inward, aligning with each other to form the core of the lipid bilayer. This arrangement minimizes contact with water and stabilizes the bilayer structure.This organized alignment results in the formation of a lipid bilayer membrane, which closely resembles the structure of biological cell membranes.

2. Lipid Bilayer Organization: The lipid bilayer forms the structural backbone of the liposome. Depending on formulation conditions, liposomes may exist as.

- **Unilamellar vesicles** – having a single lipid bilayer
- **Multilamellar vesicles** – containing multiple concentric bilayers

The bilayer encloses an aqueous core, enabling liposomes to encapsulate.

- **Hydrophilic drugs** in the aqueous interior
- **Lipophilic drugs** within the lipid bilayer

- **Amphiphilic drugs** at the bilayer interface.[17]

3. Role of Cholesterol: Cholesterol is commonly incorporated into the phospholipid bilayer to improve membrane stability. Its functions include.

- Increasing bilayer rigidity and mechanical strength.
- Reducing membrane permeability and drug leakage.
- Enhancing resistance to aggregation and fusion.
- Improving stability during storage and in biological fluids.

Cholesterol molecules intercalate between phospholipid fatty acid chains, preventing excessive fluidity or crystallization of the bilayer.[18]

4. Aqueous Core: The central aqueous compartment of liposomes provides space for the encapsulation of water-soluble drugs, peptides, proteins, and nucleic acids. The volume of the aqueous core depends on the size and lamellarity of the liposome.

5. Surface Modifications (Optional Components): In advanced formulations, the liposome surface may be modified with.[19]

- **Polyethylene glycol (PEG)** for prolonged circulation (stealth liposomes)
- **Ligands, antibodies, or peptides** for targeted drug delivery
- **Charge-inducing agents** to control surface charge and interaction with cells

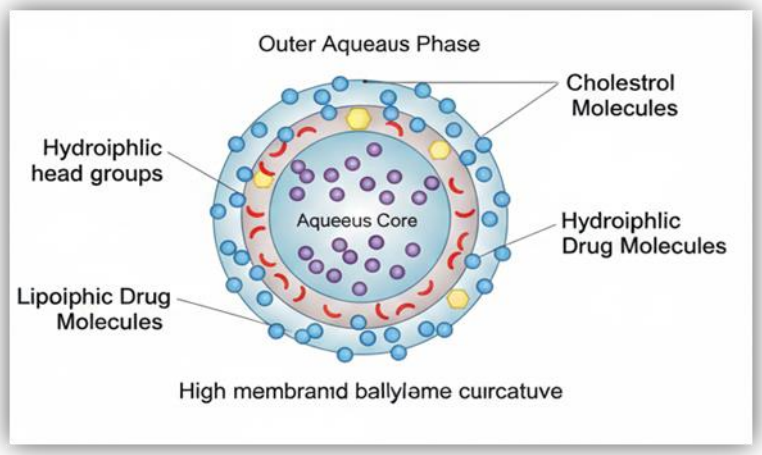

Figure I: Structure of Liposomes.

CLASSIFICATION OF LIPOSOMES

Liposomes are classified based on size, lamellarity, surface characteristics, and functional behavior**,** as these factors significantly influence drug loading, release, biodistribution, and therapeutic performance.[20]

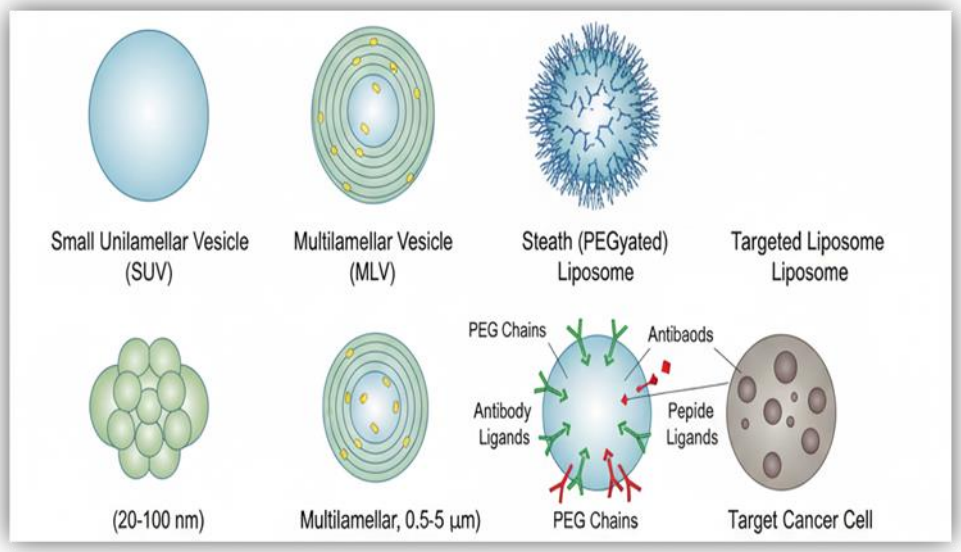

Figure II: Classification of liposomes.

1. Small Unilamellar Vesicles (SUVs): Small unilamellar vesicles consist of a single phospholipid bilayer and have a size range of 20–100 nm**.** Due to their small size, SUVs exhibit high membrane curvature, which provides better tissue penetration and cellular uptake. However, they have limited aqueous core volume, resulting in lower encapsulation efficiency for hydrophilic drugs. SUVs are commonly used for intravenous drug delivery and targeting applications**.**[21]

2. Large Unilamellar Vesicles (LUVs): Large unilamellar vesicles also possess a single lipid bilayer**,** but their size is greater than 100 nm. The larger internal aqueous volume allows higher encapsulation of hydrophilic drugs, making them suitable for proteins, peptides, and nucleic acids. LUVs provide better control over drug release compared to SUVs and are widely explored in therapeutic and diagnostic applications.

3. Multilamellar Vesicles (MLVs): Multilamellar vesicles contain multiple concentric lipid bilayers**,** resembling an onion-like structure, with sizes ranging from 0.5 to 5 µm. MLVs are relatively easy to prepare and exhibit high stability**.** They are particularly useful for

encapsulating lipophilic drugs within the multiple bilayers**,** but their large size limits intravenous use and favors topical or localized drug delivery.[22]

4. Stealth (PEGylated) Liposomes: Stealth liposomes are surface-modified with polyethylene glycol (PEG)**,** which forms a protective hydrophilic layer around the vesicle. This modification prevents recognition by plasma proteins and macrophages, thereby reducing reticuloendothelial system (RES) uptake**.** As a result, stealth liposomes have prolonged circulation time and improved drug accumulation at target sites, especially in cancer therapy.

5. Targeted Liposomes: Targeted liposomes are designed by conjugating specific ligands, antibodies, peptides, or sugars onto the liposome surface. These ligands bind selectively to receptors overexpressed on diseased cells, enabling active targeting**.** Targeted liposomes enhance therapeutic efficacy while minimizing systemic toxicity and are extensively used in anticancer and gene therapy applications**.**[23]

6. Stimuli-Responsive Liposomes: Stimuli-responsive liposomes release their drug payload in response to internal or external triggers such as pH changes, temperature, enzymes, ultrasound, or light. For example, pH-sensitive liposomes release drugs in acidic tumor or endosomal environments. These systems allow site-specific and controlled drug release**,** improving therapeutic outcomes.[24]

FORMULATION METHODS OF LIPOSOMES

The method of preparation significantly affects vesicle size, lamellarity, encapsulation efficiency, drug loading, and stability**.**

CLASSIFICATION OF LIPOSOMES

1. Thin Film Hydration Method: This is the most widely used laboratory-scale method**.** Phospholipids and cholesterol are dissolved in an organic solvent, which is evaporated under reduced pressure to form a thin lipid film on the wall of a round-bottom flask. The film is then hydrated with an aqueous drug solution, resulting in the formation of multilamellar **vesicles**. Size reduction techniques such as sonication or extrusion may be applied to obtain SUVs or LUVs.[25]

2. Reverse Phase Evaporation Method: In this method, lipids are dissolved in organic solvent and mixed with an aqueous phase containing the drug to form a water-in-oil

emulsion. Removal of the organic solvent under reduced pressure leads to the formation of liposomes with a large aqueous core**,** typically LUVs.

3. Ethanol Injection Method: Here, a lipid solution in ethanol is rapidly injected into an aqueous phase containing the drug. Ethanol diffuses into the aqueous phase, causing spontaneous formation of small unilamellar vesicles**.**

4. Ether Injection Method: In this method, a lipid solution in diethyl ether is slowly injected into a heated aqueous phase. As ether evaporates, liposomes are formed, usually producing unilamellar vesicles**.**

5. Microfluidization and High-Pressure Homogenization : These are industrial-scale methods where lipid dispersions are passed through narrow channels under high pressure, resulting in uniform vesicle size and improved reproducibility.

6. Supercritical Fluid Technique: This advanced method uses supercritical CO_2 to dissolve lipids and form liposomes without organic solvents. It produces liposomes with high purity and controlled size**.**[26]

CHARACTERIZATION OF LIPOSOMES

Characterization of liposomes is a critical step in their development, as it ensures the final formulation meets the desired quality, safety, stability, and therapeutic performance criteria. Proper characterization allows researchers to predict in vivo behavior, optimize formulation parameters, and achieve consistent drug delivery outcomes. Because liposomes are complex nanocarriers, several physicochemical, morphological, and functional properties need to be assessed.

Table I: Characterization of liposomes.

PARAMETER	PURPOSE	TECHNIQUES
Vesicle Size & Distribution	Determines biodistribution	DLS, Laser Diffraction
Zeta Potential	Predicts stability	Zeta Analyzer
Encapsulation Efficiency	Drug loading capacity	UV, HPLC
Morphology	Structural integrity	TEM, SEM
In-vitro Drug Release	Release kinetics	Dialysis method
Stability Studies	Shelf life	Accelerated stability testing

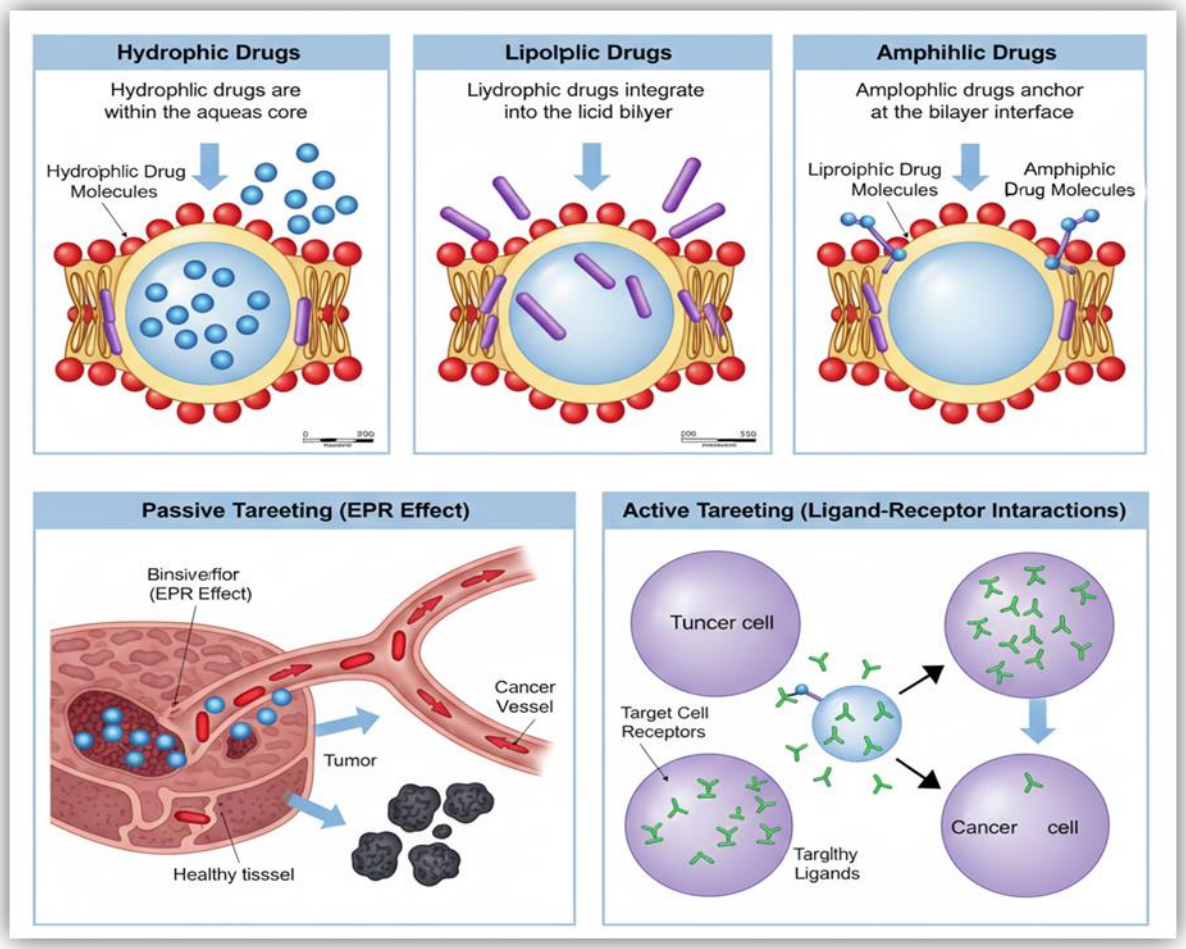

Figure III: Drug Encapsulation.

THERAPEUTIC APPLICATIONS OF LIPOSOMES

Liposomes are highly versatile nanocarriers that have been extensively explored for **various therapeutic applications** due to their ability to encapsulate both hydrophilic and lipophilic drugs, protect labile molecules, control drug release, and enable targeted delivery. Their clinical success has led to several FDA-approved formulations.

1. Cancer Therapy: Liposomes are widely used in oncology to improve efficacy and reduce **systemic toxicity** of chemotherapeutic agents. By encapsulating cytotoxic drugs, liposomes enhance tumor targeting through the enhanced permeability and retention (EPR) effect. Examples of clinically approved liposomal anticancer drugs**.**

- **Doxil® (liposomal doxorubicin):** Reduces cardiotoxicity and improves circulation time.
- **DaunoXome® (liposomal daunorubicin):** Targets cancer cells with minimized off-target effects.[23]

2. Antifungal Therapy: Conventional antifungal drugs often cause systemic toxicity, particularly nephrotoxicity in the case of amphotericin B. Liposomal formulations improve safety and therapeutic index. Example: AmBisome® (liposomal amphotericin B): Minimizes nephrotoxicity while maintaining antifungal efficacy.[22]

3. Vaccine Delivery: Liposomes act as efficient adjuvants and delivery vehicles for vaccines, improving antigen presentation and enhancing the immune response.

- Liposomal vaccines can encapsulate proteins, peptides, or nucleic acids, protecting them from degradation and ensuring controlled release.

4. Gene and siRNA Delivery: Liposomes serve as non-viral vectors for gene therapy and RNA interference (siRNA) delivery, providing safer alternatives to viral vectors.

- Liposomes can encapsulate nucleic acids, protect them from enzymatic degradation, and facilitate cellular uptake via endocytosis.[25]
- Surface modifications such as cationic lipids or PEGylation improve stability, targeting, and transfection efficiency.
- Applications include treatment of genetic disorders, cancers, and viral infections.

5. Ocular Drug Delivery: Liposomes enhance ocular drug delivery by improving drug retention, bioavailability, and corneal penetration. Liposomes can be formulated as eye drops or gels, allowing sustained release and reducing dosing frequency.

6. Pulmonary Delivery: Liposomes are explored for inhalation therapy to treat respiratory diseases.

- They protect labile drugs and improve drug deposition in the lungs, while minimizing systemic side effects.[23]
- Applications include treatment of tuberculosis, asthma, and cystic fibrosis.

7. Transdermal and Topical Delivery: Liposomes enhance skin penetration and allow controlled release of drugs through the skin barrier.[27]

- Applications include delivery of anti-inflammatory drugs, hormones, and local anesthetics.
- Liposomes improve drug solubility, stability, and bioavailability in topical formulations.

CONCLUSION

Liposomes have emerged as one of the most promising and versatile platforms in modern drug delivery systems (DDS)**,** owing to their biocompatibility, structural versatility, and ability to encapsulate both hydrophilic and lipophilic therapeutic agents**.** Their unique bilayered vesicular structure allows them to protect drugs from enzymatic and chemical

degradation, prolong systemic circulation, and facilitate controlled or targeted drug release. This feature is particularly beneficial for anticancer agents, antifungal drugs, vaccines, and gene therapy, where conventional formulations often fail due to toxicity or poor bioavailability.

The clinical success of liposomes, evidenced by FDA-approved products such as Doxil®, DaunoXome®, and AmBisome®**,** highlights their ability to enhance therapeutic efficacy while reducing systemic toxicity. Furthermore, advancements in lipid chemistry, surface modification (PEGylation), targeting ligands, and stimuli-responsive formulations have significantly expanded the scope of liposomal applications, enabling precision medicine and personalized therapeutics**.**

Despite these advantages, challenges such as physical instability, rapid RES clearance, limited drug loading, and high production costs persist. However, ongoing research in nanotechnology, microfluidic-based fabrication, and multifunctional liposomal platforms is addressing these limitations, paving the way for next-generation liposomal systems**.**

Future developments are likely to focus on smart liposomes integrated with biosensors**,** theranostic applications combining imaging and therapy, and tailored formulations for specific diseases**,** including cancer, infectious diseases, and chronic disorders. Overall, liposomes continue to represent a cornerstone of novel drug delivery, with immense potential to transform therapeutic strategies, improve patient outcomes, and redefine the landscape of nanomedicine and personalized therapy**.**

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