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A Comprehensive Review on Liposome: Preparation Methods, Characterization, And Applications

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ABSTRACT:
Liposomes have become one of the most important and well-established nanocarrier systems in pharmaceutical and biomedical research. From their early use as simple models of biological membranes, liposomes have steadily developed into clinically proven drug delivery platforms. Their capacity to carry both water-soluble and lipid-soluble drugs, along with their excellent biocompatibility and adaptable structure, has supported their wide application in drug delivery, gene therapy, vaccination, and diagnostic fields. This review brings together information from multiple review articles to present a clear and comprehensive overview of liposomal drug delivery systems. It discusses the origin and development of liposomes, their classification and composition, mechanisms of formation, traditional and modern preparation techniques, characterization methods, stability issues, targeting strategies, therapeutic applications, marketed products, and recent technological advancements. Current challenges and future directions in liposome research are also highlighted. Overall, this review aims to provide an easy-to-understand yet detailed reference for students, researchers, and professionals working in the area of liposomal drug delivery.

KEYWORDS: Liposomes; Vesicular drug delivery systems; Nanocarriers; Phospholipid bilayers; Liposome classification; Mechanism of formation; Conventional and novel preparation methods; Characterization techniques; Targeted liposomes; Stealth liposomes; Stimuli-responsive liposomes; Therapeutic applications; Nanomedicine.

I. INTRODUCTION

Liposomes are among the most widely studied and versatile vesicular systems used in modern pharmaceutical and biomedical sciences [1–4]. Since their discovery by Bangham in the 1960s, liposomes have evolved from simple experimental models of biological membranes into advanced nanocarriers with proven clinical relevance [1,2]. Structurally, liposomes are spherical vesicles composed of one or more phospholipid bilayers surrounding an aqueous core. This architecture closely mimics natural cell membranes and is responsible for their excellent biocompatibility, biodegradability, and low toxicity [3–5].

A major advantage of liposomes lies in their ability to encapsulate a wide variety of therapeutic agents. Hydrophilic drugs can be entrapped within the aqueous core, lipophilic drugs can be incorporated into the lipid bilayer, and amphiphilic drugs can be distributed between both regions [4,6]. Conventional drug delivery systems often face limitations such as poor aqueous solubility, chemical and enzymatic degradation, rapid clearance from systemic circulation, low bioavailability, and non-specific tissue distribution leading to adverse effects [7,8]. Liposomal drug delivery systems help overcome these challenges by protecting drugs from degradation, improving pharmacokinetic behavior, prolonging circulation time, and reducing toxicity to healthy tissues [2,6,9].

Furthermore, liposomes can be engineered to provide controlled and sustained drug release, enabling maintenance of therapeutic drug levels for extended durations [10]. Advances in lipid chemistry, formulation science, and surface modification techniques have led to the development of specialized liposomes, including long-circulating (stealth) liposomes, ligand-targeted liposomes, and stimuli-responsive liposomes [10–13]. These advanced systems have significantly expanded the applications of liposomes in cancer therapy, antifungal treatment, vaccine delivery, gene and nucleic acid delivery,

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immunotherapy, and diagnostic imaging [11,14–18]. Ongoing research continues to explore novel liposomal designs, reinforcing their central role in nanomedicine and next-generation drug delivery systems [2,5].

II. HISTORICAL BACKGROUND AND EVOLUTION OF LIPOSOMES

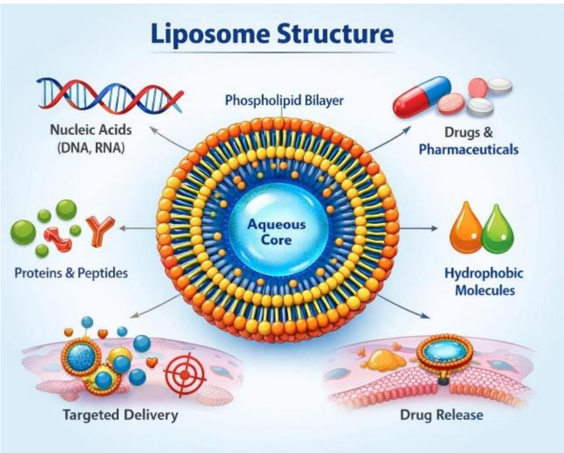
Liposomes were first described by Alec D. Bangham in 1965 during studies on phospholipid dispersions in aqueous systems [1]. Initially, liposomes were mainly used as model membranes for studying biological processes [1,3]. Early therapeutic applications were limited by instability, rapid clearance, and formulation challenges [8,13].

During the 1970s and 1980s, the potential of liposomes as drug carriers became evident, with studies demonstrating improved therapeutic efficacy and reduced toxicity of encapsulated drugs [9,14]. Continuous advances in lipid composition, preparation methods, and surface modification strategies led to clinically viable formulations. The approval of liposomal doxorubicin marked a major milestone in liposomal drug delivery [17,18].

III. STRUCTURE COMPOSITION AND CLASSIFICATION OF LIPOSOMES.

Liposomes exhibit significant structural and functional diversity, which allows them to be tailored for specific therapeutic and diagnostic applications. Based on their composition, size, lamellarity, surface charge, and functional characteristics, liposomes can be broadly classified into several categories.

3.1 Structural Components



Liposomes are mainly composed of natural or synthetic phospholipids, which are amphiphilic molecules containing a hydrophilic head group and hydrophobic fatty acid chains [3–5]. Commonly used phospholipids include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylglycerol. Cholesterol is frequently incorporated into the lipid bilayer to improve membrane rigidity, reduce permeability, and enhance stability in biological environments [14]. Other components such as glycolipids, sphingolipids, and membrane proteins may also be included to impart specific functional properties.

3.2 Classification Based on Lamellarity and Size

Based on the number of lipid bilayers and vesicle size, liposomes are classified as:

Multilamellar vesicles (MLVs): These consist of multiple concentric bilayers and typically range from 0.5 to several micrometers in size. They are relatively easy to prepare and offer high drug loading capacity.

Small unilamellar vesicles (SUVs): These contain a single lipid bilayer and generally have diameters between 20–100 nm. SUVs are suitable for systemic delivery due to their small size.

Large unilamellar vesicles (LUVs): These vesicles have a single bilayer with sizes ranging from 100–1000 nm and provide improved encapsulation efficiency for hydrophilic drugs [4,15].

3.3 Classification Based on Surface Charge

Depending on the lipid composition, liposomes may be:

Neutral liposomes, which exhibit minimal interaction with biological components

Cationic liposomes, which interact strongly with negatively charged cell membranes and are widely used for gene delivery

Anionic liposomes, which offer improved stability and reduced toxicity in certain applications [6,11]

Surface charge plays a critical role in determining circulation time, cellular uptake, biodistribution, and stability.

3.4 Classification Based on Composition

Liposomes can also be classified based on their lipid composition:

Conventional liposomes, composed mainly of phospholipids and cholesterol

Stealth liposomes, containing polyethylene glycol (PEG) on the surface to evade reticuloendothelial system uptake and prolong circulation time

Cationic liposomes, formulated with positively charged lipids for nucleic acid delivery

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3.5 Classification Based on Functional Behavior

Based on their functional properties, liposomes are categorized as:

Targeted liposomes, modified with ligands such as antibodies, peptides, or sugars for site-specific delivery

Stimuli-responsive liposomes, which release drugs in response to pH, temperature, enzymes, light, or magnetic fields

Long-circulating liposomes, designed to remain in systemic circulation for extended periods

Theranostic liposomes, combining therapeutic and diagnostic agents in a single system [10–13,25]

This broad classification highlights the versatility of liposomes and their ability to be customized for diverse biomedical applications.

IV. MECHANISM OF LIPOSOMES FORMULATION

The formation of liposomes is a spontaneous and energetically favorable process driven by the amphiphilic nature of phospholipid molecules in an aqueous environment [3,5]. Phospholipids consist of hydrophilic (water-attracting) head groups and hydrophobic (water-repelling) fatty acid tails. When dispersed in water, these molecules self-assemble in a manner that minimizes free energy by shielding the hydrophobic tails from the aqueous phase while allowing the hydrophilic heads to interact with water.

Upon hydration, phospholipid molecules initially form planar bilayer sheets in which the hydrophobic tails face inward and the hydrophilic heads face outward toward the surrounding aqueous medium [3]. However, these bilayer sheets are thermodynamically unstable due to the exposure of hydrophobic edges to water. To reduce this unfavorable interaction, the bilayers curve and eventually close upon themselves, forming spherical vesicular structures known as liposomes.

The formation and characteristics of liposomes are strongly influenced by several factors, including lipid composition, temperature, hydration medium, and the presence of additives such as cholesterol [5,14]. The phase transition temperature of phospholipids plays a critical role, as liposome formation is most efficient when lipids are hydrated above their transition temperature, where the bilayer is in a fluid state. Cholesterol intercalates between phospholipid molecules, modulating membrane fluidity, permeability, and stability.

External energy input is often required to facilitate liposome formation and control vesicle size and lamellarity. Techniques such as mechanical agitation, vortexing, sonication, homogenization, extrusion, and microfluidization provide the necessary energy to break down larger lipid aggregates into smaller, more uniform vesicles [22,23]. These processes influence whether multilamellar, unilamellar, or nanosized liposomes are formed.

In addition to physical factors, the nature of the encapsulated drug also affects liposome formation and loading efficiency. Hydrophilic drugs are entrapped within the aqueous core during hydration, whereas lipophilic drugs partition into the lipid bilayer. Amphiphilic drugs may localize at the bilayer interface [4,6]. Overall, the mechanism of liposome formation is a complex interplay of molecular self-assembly, thermodynamics, and external processing conditions, which together determine the final properties and performance of the liposomal system.

V. CONVENTIONAL METHODS OF LIPOSOME PREPARATION

Conventional methods of liposome preparation are widely used due to their simplicity, versatility, and suitability for laboratory-scale formulations. These methods primarily rely on the hydration of lipid films or lipid solutions in an aqueous medium, followed by size reduction processes [4,7]. The choice of method significantly influences vesicle size, lamellarity, encapsulation efficiency, and stability.

5.1 Thin Film Hydration Method (Bingham Method)

The thin film hydration method is the most classical and extensively used technique for liposome preparation. In this method, lipids are dissolved in a suitable organic solvent such as chloroform or methanol. The solvent is then evaporated under reduced pressure using a rotary evaporator, resulting in the formation of a thin, dry lipid film on the walls of the flask. Hydration of this film with an aqueous phase above the lipid phase transition temperature leads to swelling and spontaneous formation of multilamellar vesicles (MLVs) [1,3]. This method is simple and versatile but often requires further processing such as sonication or extrusion to reduce particle size.

5.2 Reverse Phase Evaporation Method

In the reverse phase evaporation method, lipids are dissolved in an organic solvent and mixed with an aqueous phase to form a water-in-oil emulsion using sonication or homogenization.

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Removal of the organic solvent under reduced pressure leads to the formation of liposomes with a large internal aqueous space, making this method particularly suitable for encapsulating hydrophilic drugs with high efficiency [6,15]. However, exposure to organic solvents and mechanical stress may limit its applicability for sensitive biomolecules.

5.3 Ether and Ethanol Injection Methods

In ether injection, a lipid solution in diethyl ether is slowly injected into a warm aqueous phase, leading to rapid solvent evaporation and liposome formation. Similarly, in the ethanol injection method, lipids dissolved in ethanol are injected into an aqueous medium, producing small unilamellar vesicles [7,8]. These methods are relatively simple and do not require sophisticated equipment, but residual solvent and limited encapsulation efficiency may be disadvantages.

5.4 Detergent Removal Method

This method involves the solubilization of lipids using detergents to form mixed micelles, followed by gradual removal of the detergent through dialysis or adsorption. As the detergent is removed, liposomes are formed. This method is particularly useful for incorporating membrane proteins into liposomes [5].

VI. NOVEL AND ADVANCED METHODS OF LIPOSOME PPREPARATION

To overcome the limitations associated with conventional techniques, several novel and advanced liposome preparation methods have been developed. These methods aim to improve scalability, reproducibility, size control, and encapsulation efficiency while minimizing solvent exposure [11,22].

6.1 Microfluidization and High-Pressure Homogenization

Microfluidization involves forcing lipid and aqueous phases through microchannels at high pressure, resulting in uniform and nanosized liposomes. This method offers excellent batch-to-batch reproducibility and is suitable for large-scale production [23,35].

6.2 Supercritical Fluid Technology

Supercritical carbon dioxide is used as a solvent or antisolvent to form liposomes without the use of toxic organic solvents. This environmentally friendly approach allows precise control over particle size and lipid composition [22].

6.3 Freeze–Thaw Method

Repeated freezing and thawing of liposomal dispersions promotes fusion of vesicles and improves encapsulation efficiency, particularly for macromolecules such as proteins and nucleic acids [4].

6.4 Microfluidic-Based Liposome Preparation

Microfluidic systems enable precise control over mixing conditions at the micro scale, allowing the production of highly uniform liposomes with tunable sizes. These systems are increasingly explored for personalized medicine applications [25].

VII. APPLICATIONS OF LIPOSOMES

Liposomes have been extensively investigated and successfully applied across a wide range of therapeutic and diagnostic fields due to their versatility and safety profile [11]. In cancer chemotherapy, liposomal formulations enhance drug accumulation at tumor sites, reduce systemic toxicity, and improve patient tolerability, as demonstrated by several clinically approved products [17,18]. In antifungal therapy, liposomal formulations such as amphotericin B significantly reduce nephrotoxicity while maintaining therapeutic efficacy [24].

Liposomes are also widely used in antimicrobial and antiviral drug delivery, where they improve drug stability and intracellular delivery [11]. In vaccine delivery, liposomes function as effective adjuvants and antigen carriers, enhancing immune responses [14]. Cationic liposomes have gained importance in gene therapy and nucleic acid delivery, facilitating cellular uptake of DNA, RNA, and siRNA [16]. Additionally, liposomes are increasingly employed in diagnostic imaging and theranostic applications, where they enable simultaneous disease diagnosis and treatment [5,25].

VIII. RECENT ADVANCEMENTS IN LIPOSOME TECHNOLOGY

Recent advancements in liposome technology focus on the development of multifunctional and intelligent delivery systems capable of addressing complex therapeutic needs [5,11]. These include stimuli-responsive liposomes that release their payload in response to specific triggers such as pH, temperature, enzymes, or magnetic fields [10,25]. Combination therapy using liposomes loaded with multiple drugs has also gained attention for synergistic treatment effects, particularly in cancer therapy.

Further progress has been made in the field of personalized medicine, where liposomal formulations are tailored based on individual patient requirements [11]. The integration of therapeutic and diagnostic agents into a single liposomal carrier, known as theranostic liposomes, represents a promising approach for real-time monitoring of treatment efficacy [5]. Advances in large-scale manufacturing and quality control are also contributing to the wider clinical translation of novel liposomal systems [23,35].

IX. CHALLENGES AND LIMITATIONS

Despite the numerous advantages and successful clinical applications of liposomes, several challenges and limitations continue to restrict their broader use [8,23,35]. One of the major challenges is formulation stability. Liposomes are susceptible to physical and chemical instability, including aggregation, fusion, leakage of encapsulated drugs, phospholipid oxidation, and hydrolysis during storage. These stability issues can significantly affect shelf life and therapeutic performance.

Large-scale manufacturing and reproducibility also remain critical limitations. Many liposome preparation methods that work well at the laboratory scale are difficult to translate to industrial production while maintaining consistent particle size, encapsulation efficiency, and batch-to-batch uniformity. High production costs associated with raw materials, specialized equipment, and stringent quality control further limit widespread commercialization.

Another major challenge is rapid clearance by the reticuloendothelial system, which can reduce circulation time and therapeutic efficacy, particularly for conventional liposomes. Although surface modification strategies such as PEGylation have addressed this issue to some extent, they may introduce new concerns such as immunogenic reactions and accelerated blood clearance upon repeated administration. Additionally, regulatory approval of liposomal formulations is complex due to their structural complexity, making characterization, quality assurance, and bioequivalence assessment more challenging than conventional dosage forms.

X. FUTURE PERSPECTIVE

The future of liposomal drug delivery is highly promising, driven by continuous advancements in nanotechnology, materials science, and molecular biology [5,11,25]. Future research efforts are expected to focus on the development of smarter and more sophisticated liposomal systems capable of precise targeting, controlled drug release, and enhanced therapeutic efficacy. Stimuli-responsive liposomes that release drugs in response to specific internal or external triggers such as pH, enzymes, temperature, light, or magnetic fields are likely to gain increased clinical attention.

Personalized medicine represents another important future direction, where liposomal formulations may be tailored according to individual patient characteristics, disease states, and genetic profiles. The integration of therapeutic and diagnostic agents into a single carrier system, known as theranostic liposomes, holds great potential for real-time monitoring of disease progression and treatment response. Furthermore, advancements in large-scale manufacturing technologies and regulatory frameworks are expected to facilitate smoother clinical translation and commercialization of novel liposomal formulations.

Overall, liposomes are expected to play an increasingly significant role in next-generation drug delivery systems, contributing to safer, more effective, and patient-specific therapies.

XI. CONCLUSION

Liposomes have emerged as one of the most versatile and clinically successful nanocarrier systems in modern drug delivery and nanomedicine. Their unique structural features, biocompatibility, and ability to encapsulate a wide range of therapeutic agents have enabled significant improvements in drug efficacy, safety, and patient compliance. Over the years, continuous advancements in liposome formulation, surface modification, and preparation technologies have expanded their applications from conventional drug delivery to targeted therapy, gene delivery, vaccination, and diagnostic imaging.

Despite existing challenges related to stability, manufacturing, cost, and regulatory complexity, ongoing research and technological innovation continue to address these limitations. The successful approval of several liposomal products highlights their translational potential and clinical relevance. With continued interdisciplinary research and improved industrial scalability, liposomes are expected to remain a cornerstone of advanced drug delivery systems. Ultimately, liposomal technology holds great promise for shaping the future of personalized medicine and improving therapeutic outcomes across a wide range of diseases.

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