Review On: Targeted Liposomal Drug Delivery System

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***Abstract*—Targeted liposomal drug delivery systems are innovative therapeutic strategies designed to enhance the specificity and efficacy of drug treatments. These systems involve the encapsulation of therapeutic agents within liposomes—nano-sized lipid-based vesicles—and functionalizing the liposome surface with targeting ligands. These ligands, such as antibodies or peptides, enable the liposomes to selectively bind to specific cells or tissues, often those involved in diseases like cancer, thereby improving drug accumulation at the desired site of action. This targeted approach minimizes off-target effects, reduces systemic toxicity, and improves the bioavailability of the drug. Liposomal drug delivery systems also offer controlled release profiles, further enhancing therapeutic outcomes. Consequently, they offer great potential for enhancing the accuracy and efficiency of various treatments, especially in cancer therapy and other specialized interventions.**

***Key Words*—Targeted liposomes, drug delivery, cancer therapy, nanomedicine, personalized medicine**

INTRODUCTION

When dispersed in water, phospholipids naturally organize into closed structures with an internal aqueous environment surrounded by phospholipid bilayer membranes. These structures are known as liposomes [1]. Liposomes are round vesicles that can be created using cholesterol, non-toxic surfactants, sphingolipids, glycolipids, long-chain fatty acids, and membrane proteins [2]. Acting as drug carriers, liposomes are capable of encapsulating a wide range of molecules, including small drug compounds, proteins, nucleotides, and plasmids. First discovered approximately 40 years ago by A.D. Bangham [3], liposomes have since become valuable tools in biology, biochemistry, and medicine. By the 1960s, liposomes were employed as carriers to deliver various substances within their aqueous compartments. They can be designed and modified to vary in size, composition, charge, and lamellarity. Today, several liposomal formulations of anti-tumor and antifungal drugs have been successfully commercialized [4].

Targeted drug delivery systems enhance the pharmacological properties of drugs by modifying their pharmacokinetics and biodistribution. Tumor-specific delivery systems are particularly valuable in cancer therapy, where the efficacy of chemotherapeutics is often hindered by severe side effects. Liposomal drug delivery systems (DDS) are widely utilized for delivering anti-cancer agents, with several drugs successfully encapsulated, including doxorubicin, daunorubicin, annamycin, cisplatin derivatives, vincristine, paclitaxel, camptothecin derivatives, 5-fluorouracil derivatives, and retinoids. Using remote-loading techniques, anthracyclines and other agents can achieve nearly 100% trapping efficiency within pre-formed liposomes [20]. Additionally, liposomes are employed for delivering macromolecules such as superoxide dismutase, hemoglobin, tumor necrosis factor, erythropoietin, interleukin-2, and interferon-γ [13].

Liposomal formulations can be delivered through various administration routes, including intravenous, oral and intranasal methods. Additionally, liposomes encapsulating acyclovir have been used for ocular absorption, while vitamin E-containing liposomes have been applied to the eyes to prevent cataractogenesis [15].

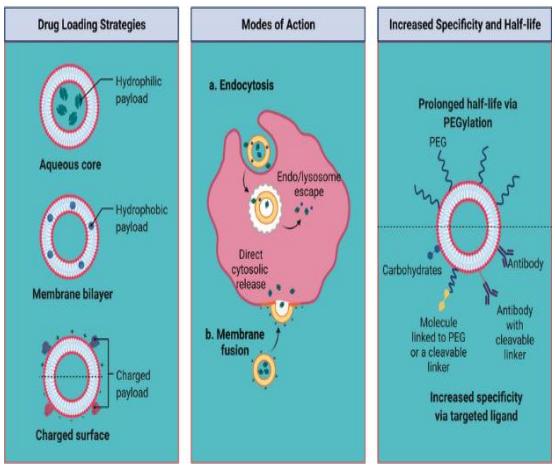

Fig I. Liposomal drug delivery systems

A schematic representation of various types of liposomal drug delivery systems [11]:

1. Conventional liposomes feature a lipid bilayer enclosing aqueous compartments, made of phospholipids and cholesterol without any surface modification.
2. PEGylated liposomes have a hydrophilic polyethylene glycol (PEG) coating on their surface, providing steric stabilization and altering there in vivo behavior and characteristics.
3. Ligand-targeted liposomes are designed to specifically interact with targets through ligands attached to the surface or the terminal ends of the PEG chains.
4. Theranostic liposomes integrate multiple functionalities into a single system, combining a nanoparticle, targeting elements, imaging capabilities, and therapeutic agents.

Liposomes consist of an outer lipid bilayer and a core that can encapsulate either hydrophobic or hydrophilic drugs, marking them as the first nano-scale drug delivery systems approved for clinical use. By modifying the lipid bilayer structure, liposomes can be tailored for specific functions, such as mimicking the biophysical properties of living cells, which enhances their ability to deliver drugs to targeted sites effectively. Amphiphilic drugs can be encapsulated within the inner aqueous core of liposomes using methods such as the ammonium sulfate gradient technique [14].

CLASSIFICATION OF LIPOSOMES

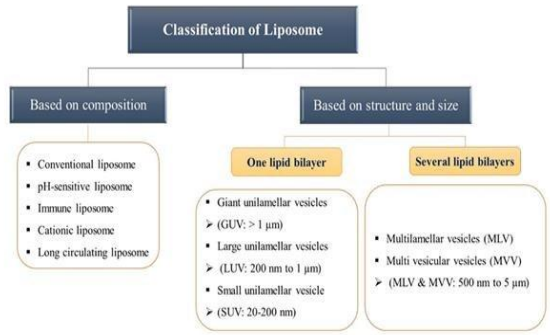

Fig II. Schematic representation of classification of liposomes

ADVANTAGES

1. Provide controlled drug delivery.
2. Biodegradable, biocompatible, flexible.
3. Liposomes have the capability to transport both water-soluble and lipid-soluble drugs.
4. Drugs can be stabilized from oxidation.
5. Improve protein stabilization.
6. Provide sustained release.
7. Facilitates targeted or site-specific drug delivery.
8. Alter pharmacokinetics and pharmacodynamics of the drug.

DISADVANTAGES

1. The development and production of liposomes involve high costs.
2. Stability Issues.
3. Complex Manufacturing Process.
4. Limited Drug Loading Capacity.
5. Short Circulation Time Without Modification.
6. Potential for Immune Reactions.
7. Difficulty in Achieving Specific Targeting.
8. Drug Leakage and Premature Release.
9. Regulatory and Approval Challenges.
10. Limited Penetration into Certain Tissues.

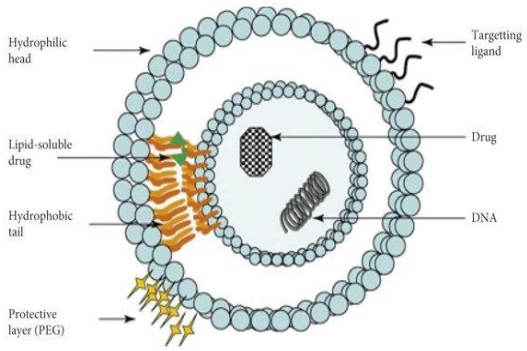

Fig III. Structure of liposomes

A diagram showing a liposome encapsulating hydrophilic drugs within its aqueous core and hydrophobic drugs within its lipid bilayer. The liposome's surface can be modified with targeting ligands and a polyethylene glycol (PEG) coating to facilitate active and passive targeting, respectively [9].

METHODS OF LIPOSOME PREPARATION

Liposomes with varying sizes and properties often require distinct preparation techniques. The simplest and most commonly used method for creating multilamellar vesicles (MLVs) is the thin-film hydration technique. In this approach, an aqueous buffer at a temperature suited to the lipids is used. For hydrophilic drugs, the drug is added to the aqueous hydration buffer, while for lipophilic drugs, it is incorporated into the lipid film. This method typically generates a heterogeneous population of MLVs with diameters ranging from 1 to 5 micrometers. These can be further processed through

sonication or extrusion using polycarbonate filters to obtain small unilamellar vesicles (SUVs) with more uniform sizes, measuring up to 0.025 micrometers [22].

A critical factor to consider in liposome preparation is the rigidity of the bilayers. Various categories of phospholipids can be utilized for this process, including [18]:

1. Phospholipids derived from natural sources
2. Phospholipids modified from natural origins
3. Semi-synthetic phospholipids
4. Fully synthetic phospholipids
5. Phospholipids with natural head groups

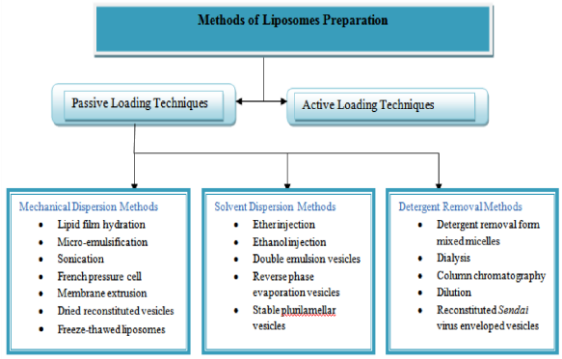

Fig IV. Schematic representation of various methods for preparation of liposomes

ACTIVE LOADING TECHNIQUE

In the active loading method, drug incorporation is achieved by establishing diffusion gradients between the internal and external aqueous phases [5]. This technique involves coating lipids and drugs onto a soluble carrier to create a free-flowing granular component in proliposomes, which forms an isotonic liposomal solution upon hydration. The pro-liposome approach provides a cost-effective and scalable method for manufacturing liposomes, particularly for incorporating lipophilic drugs [7].

PASSIVE LOADING TECHNIQUES

In the passive loading method, the drug is incorporated by adding the drug solution either prior to or during the liposome preparation. This technique is further divided into three main categories: the mechanical dispersion method, the solvent dispersion method, and the detergent removal method [5].

A. Mechanical dispersion method

1. Lipid Flim Hydration Technique

This is one of the most commonly used and straightforward methods for liposome preparation. The lipid components are first dissolved in a suitable solvent and transferred to a round-bottom flask. The organic solvent is then evaporated using a rotary evaporator connected to a vacuum pump, with the flask rotating at an optimal speed while maintaining a temperature of approximately 30°C. As a result, a thin lipid film forms on the inner walls of the flask. The rotation is continued for an additional 15 minutes after the lipid residue appears to ensure thorough drying. Afterward, the flask is detached from the evaporator, and nitrogen gas is introduced. Any remaining solvent is removed by lyophilization. Following this, nitrogen is again flushed into the flask, and a buffer is added to solubilize the lipid film. The resulting suspension contains multilamellar vesicles (MLVs) liposomes. However, this method has notable limitations, including low drug encapsulation efficiency, challenges in scaling up the process, and the production of liposomes with heterogeneous size distributions [16].

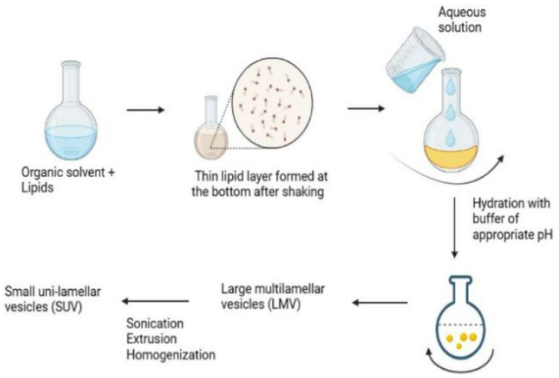

Fig V. Lipid film hydration method

2. Microemulsification

This method is commonly utilized for large-scale production of small unilamellar vesicles (SUVs). The preparation involves two key steps:

Inner Lipid Layer Formation: The inner lipid layer is prepared using a water-in-oil (w/o) microemulsion technique. Surfactant (phosphatidylcholine) and cosurfactant (Cremophor EL) are combined in various weight ratios to create a mixture referred to as "Comix." A total of 10 mL of diethyl ether (oil phase) is thoroughly mixed with the Comix in separate glass vials. The mixture is gradually diluted with a BSA (bovine serum albumin) solution (water phase), added dropwise until the solution transitions from transparent to a light blue opalescent appearance.

Outer Lipid Layer Formation: The outer lipid layer is composed of DOPE(dioleoyl- phosphoethanolamine) and DC-Chol ((dimethylaminoethane) carbamoyl cholesterol) in a 4:1 weight ratio. This layer is hydrated using 1.5 mL of a mixture of ethanol and deionized water (1:2, v/v) and incubated at 60°C for 30 minutes to form micelles.

Finally, the inner and outer lipid layers are combined in a round-bottom flask. Due to a change in solvent polarity, the micelles undergo a structural transformation, flipping to form an arrangement with hydrophilic groups oriented inward and lipophilic groups outward, ultimately leading to the formation of SUVs [17].

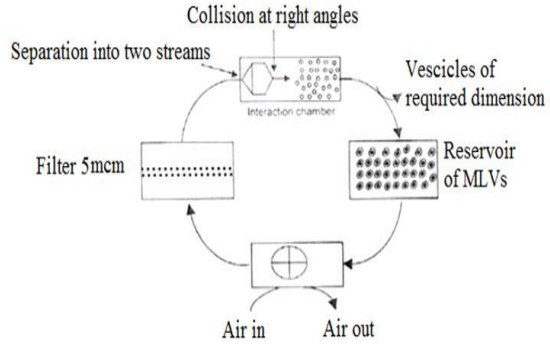

Fig VI. Microemusification of liposomes

3. Sonication

Sonication is a widely used technique for preparing small unilamellar vesicles (SUVs). In this method, multilamellar vesicles (MLVs), typically prepared via the film hydration method, are subjected to sonication to reduce their size. This process can be carried out using either a probe sonicator or a bath sonicator. The main advantage of sonication is its efficiency, as it requires relatively less time compared to other methods. However, it has several drawbacks, including low internal volume and encapsulation efficiency, the risk of metallic contamination from the probe, and the presence of residual MLVs alongside the SUVs [24].

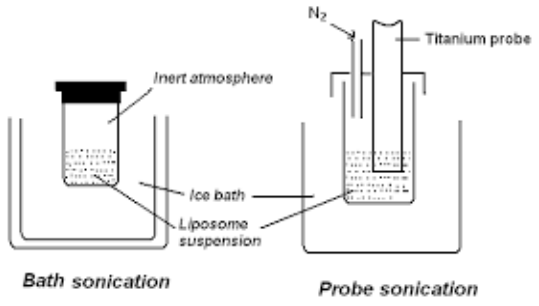

Fig VII. Bath sonication and Probe sonication method

Sonication for liposome preparation can be performed using two techniques:

Probe sonication: In this method, the tip of a sonicator is directly immersed into the lipid dispersion. This approach involves high energy dissipation, which can significantly raise the temperature of the dispersion. To mitigate this, the vessel containing the dispersion is typically placed in an ice or water bath to regulate temperature.

Bath sonication: Here, the lipid dispersion is placed in a beaker, which is then immersed in a sonication bath. This method offers better control over the temperature of the lipid dispersion compared to probe sonication. After sonication, the material can be stored in a sterile container under an inert atmosphere to maintain its integrity.

Both techniques are effective, but each has distinct considerations regarding temperature control and lipid stability.

4. French Pressure Cell Technique

Small unilamellar vesicles (SUVs) are prepared using the extrusion method by forcing multilamellar vesicles (MLVs) through a narrow orifice under conditions of 20,000 psi pressure and a temperature of 4°C.During a single pass, approximately 70% of the extruded lipids are converted into a uniform population of single bilayer vesicles. By recycling the lipid through the French pressure cell, up to 90% of the lipids can be transformed. The resulting liposomes typically range in size from 150 to 300 Å [13].

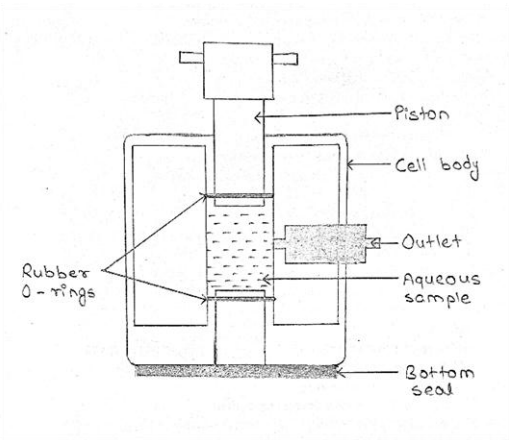

Fig VIII. French pressure cell method

Advantages:

a. The method is straightforward, quick, and highly consistent.

b. It produces liposomes larger than those obtained through sonication.

Disadvantages:

a. Temperature regulation during the process can be challenging.
b. The batch size is limited to a maximum of 50 mL, making it less suitable for large-scale production.

5. Membrane Extrusion

To obtain liposomes with a uniform size distribution, a suspension of liposomes with varying sizes is filtered through a polymer membrane. This membrane has a tortuous-path capillary pore structure due to its web-like construction. The membrane typically has a thickness of around 100 microns. The resulting liposomes have a uniform size distribution, with an average size of approximately 0.4 microns [15].

6. Freeze-thawed Liposomes

In this method, small unilamellar vesicles (SUVs) are first frozen rapidly, followed by slow thawing. During this process, the aggregation of liposomes occurs, and sonication is used to disperse the aggregated materials, resulting in the formation of large unilamellar vesicles (LUVs). The fusion of SUVs during the freezing and thawing steps leads to the creation of unilamellar vesicles (ULVs). The fusion of SUVs can be avoided by adjusting the medium's ionic strength or increasing the phospholipid concentration. The entrapment efficiency achieved with this method typically ranges from 20% to 30% [15].

B. Solvent dispersion method

1. Ether Injection

In this method, lipids are dissolved in diethyl ether or an ether/methanol mixture, which is then heated to a temperature of 55-65°C. Afterward, the lipid solution is slowly injected into an aqueous solution containing the drug or material to be encapsulated. Liposomes are formed once the ether is removed under vacuum. The main disadvantage of this method is that the liposomes produced tend to be heterogeneous in size, ranging from 70 to 190 nm. Additionally, the material being encapsulated is exposed to higher temperatures, which may not be suitable for thermolabile (heat-sensitive) drugs [19].

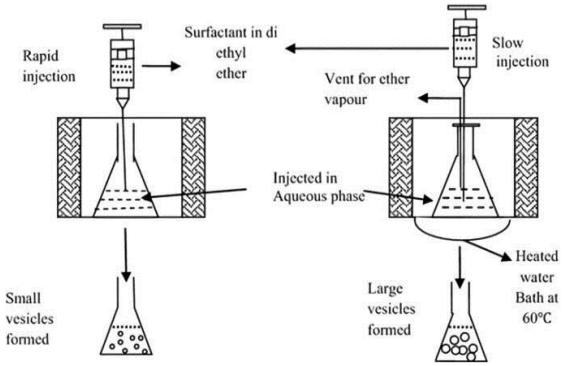

Fig IX. Ether injection method

2. Ethanol Injection

In this method, an ethanolic solution of lipids is rapidly injected into an excess of buffer, which causes the immediate formation of multilamellar vesicles (MLVs) [18].

The major drawbacks of this technique include:

a. The resulting liposome population is heterogeneous, with sizes ranging from 30 to 110 nm.
b. The azeotropic nature of ethanol with water makes it difficult to completely remove residual solvent.
c. Even low amounts of ethanol can lead to the inactivation of biologically active macromolecules encapsulated in the liposomes.

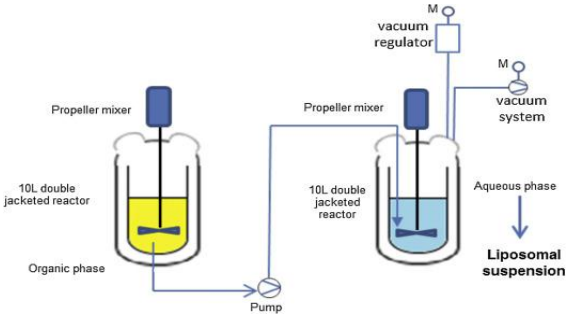

Fig X. Ethanol injection method

3. Reverse Phase Evaporation Vesicles

In this method, a lipid mixture is placed in a flask, and the solvent is removed using a rotary evaporator under reduced pressure. Under an inert atmosphere (typically using nitrogen), the lipids are redissolved in the organic phase, often using solvents such as diethyl ether or isopropyl ether. Reverse-phase vesicles are formed in this organic phase. After redispering the lipids, the aqueous phase containing the drug to be encapsulated is introduced. A one-

phase dispersion is obtained by sonication under continuous nitrogen flow. The organic solvent is then removed using the rotary evaporator until a gel-like structure forms. The resulting liposomes are known as reverse-phase evaporation vesicles (REV) [19].

CHARACTERIZATION OF LIPOSOMES

Liposomal formulations are characterized using various physicochemical and biological parameters in addition to their stability and drug entrapment efficiency. Liposomes must be characterized both after preparation and before being used in immunoassays [23].

Evaluation can be divided into three main categories:

a. Physical characterization

Physical methods involve assessing criteria such as shape, size, surface characteristics, lamellarity, phase behaviors, and drug release profiles [23].

Table I. Physical characterization of liposomes

Characterization parameter	Instrument for analysis
Vesicle shape, and surface morphology	TEM and SEM
Vesicle size and size distribution	Dynamic light scattering ,TEM
Surface charge	Free flow electrophoresis
Electrical surface potential and surface PH	Zeta potential measurement and PH sensitive probes
Lamellarity	P31NMR
Phase behaviour	DSC, freeze fracture electron microscopy
Percent capture	Mini column centrifugation, gel exclusion
Drug release	Diffuse cell/ dialysis

b. Chemical characterization

Chemical characterization focuses on research that determines the purity and effectiveness of various liposomal components. Chemical characterization includes studies that determine the purity and potency of the various components of liposomes [23].

Table II. Chemical characterization of liposomes

Characterization parameter	Instrument for analysis
Phospholipids concentration	HPLC/Barrlet assay
Cholesterol concentration	HPLC / cholesterol oxide assay
Drug concentration	Assay method
Phospholipids per oxidation	UV observance
Phospholipids hydrolysis	HPLC/ TLC
Cholesterol auto-oxidation	HPLC/ TLC
Anti-oxidant degradation	HPLC/TLC
PH	PH meter
Osmolarity	Osmometer

c. Biological characterization

Biological characterization helps analyze a formulation's efficacy and suitability for in vivo pharmacological applications. Biological characterization is essential for determining the safety of a formulation for in vivo therapeutic applications [23].

Table III. Biological characterization of liposomes

Characterization parameters	Instrument for analysis
Sterility	Aerobic/anaerobic culture
Pyrogenicity	Rabbit fever response
Animal toxicity	Monitoring survival rats

APPLICATION OF LIPOSOMES

The applications of liposomes can be seen in diagnosis and treatment as well. The various applications of liposomes are illustrated in Fig. (XI).

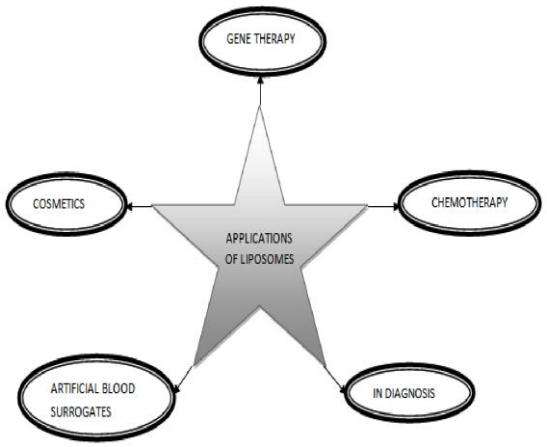

Fig XI. Schematic representation of Application of Liposomes

1. Liposomes in Cancer Therapy
2. Liposome in Gene Therapy
3. Liposomes as Vaccine System

4. Liposome as Artificial Blood Surrogates
5. Liposomes in Cosmetics and Dermatology
6. Liposomes in Diagnosis

CONCLUSIONS

Liposomes are regarded as efficient carriers in drug delivery systems. Their removal from circulation primarily occurs through trapping in the reticuloendothelial system following opsonization by serum proteins. To address this, liposomes with reduced opsonization have been designed, offering extended circulation times and the ability to accumulate in tumors due to the leaky vasculature of tumor tissues. Assessing the in vivo behavior of liposomes is crucial for their practical application in drug delivery, as this behavior is significantly influenced by the composition and concentration of the lipids within the liposomes [13].

Targeted liposomal drug delivery systems represent a significant advancement in modern therapeutics. By combining the biocompatibility and encapsulation efficiency of liposomes with targeting strategies, these systems can enhance the precision and efficacy of drug delivery while minimizing off-target effects. Innovations such as surface modification, ligand attachment, and reduced opsonization have enabled liposomes to achieve extended circulation times and preferential accumulation in target tissues, such as tumors. However, the clinical translation of these systems requires careful optimization of lipid composition, stability, and targeting mechanisms. With ongoing research, targeted liposomal drug delivery holds promise for addressing complex medical challenges and improving patient outcomes in a variety of diseases [17].

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