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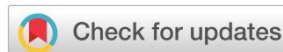
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Review Article

Next-Generation Liposomes for Drug Delivery: Engineering Strategies and Therapeutic Applications

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Abstract

Liposomes are versatile nanocarrier systems widely investigated for pharmaceutical drug delivery because of their biocompatibility, biodegradability, and ability to encapsulate both hydrophilic and lipophilic drugs. Since their discovery in the 1960s, liposomes have evolved into advanced nanomedicine platforms with applications in cancer therapy, antimicrobial treatment, gene delivery, vaccines, and personalised medicine. Advances in lipid chemistry, nanotechnology, and pharmaceutical engineering have enabled the development of long-circulating, targeted, stimuli-responsive, and multifunctional liposomal formulations. Several liposome-based products have received regulatory approval, demonstrating their clinical and translational potential. However, challenges related to formulation stability, encapsulation efficiency, large-scale manufacturing, storage, and regulatory requirements remain. Emerging approaches, including microfluidics, artificial intelligence-assisted formulation development, precision nanomedicine, and personalised drug delivery, are expected to address these limitations. This review discusses the historical development, structural characteristics, classification, preparation methods, therapeutic applications, clinical status, and future perspectives of liposomal drug delivery systems, with particular emphasis on recent innovations driving the development of next-generation liposomal therapeutics.

Keywords: Liposome, Nanomedicine, Drug Delivery System, Phospholipid Vesicles, Targeted Drug Delivery, Nanotechnology, Lipid Nanocarriers, Controlled Drug Release.

INTRODUCTION

Drug delivery has undergone substantial transformation over the past several decades, moving from conventional dosage forms towards advanced nanotechnology-based delivery systems capable of improving therapeutic outcomes while minimising adverse effects. Conventional drug administration frequently encounters limitations such as poor aqueous solubility, low bioavailability, rapid systemic elimination, non-specific tissue distribution and dose-related toxicity. These shortcomings often compromise therapeutic efficacy and patient compliance, particularly in the treatment of chronic diseases such as cancer, infectious diseases, autoimmune disorders and neurological conditions. Consequently, researchers have devoted considerable attention to developing novel carrier systems capable of overcoming these challenges and enhancing the precision of drug delivery¹.

Among the numerous nanocarrier systems developed to date, liposomes represent one of the earliest and most successful examples of clinically translated

nanomedicine. Liposomes are spherical vesicles composed primarily of phospholipid bilayers surrounding an aqueous core.²

The rapid growth of nanotechnology has further expanded the potential applications of liposomal drug delivery⁴. Innovations in molecular engineering, biotechnology and pharmaceutical manufacturing have enabled the production of highly sophisticated liposomal systems with improved stability, reproducibility and scalability. Modern manufacturing approaches such as microfluidics, continuous-flow production and quality-by-design methodologies have significantly improved formulation consistency while reducing production costs. These technological advances have accelerated the translation of liposomal formulations from laboratory research into clinical practice⁵.

In addition to therapeutic applications, liposomes have become valuable research tools in molecular biology, diagnostic imaging, vaccine development and gene therapy. Their ability to interact with biological

membranes and deliver diverse therapeutic payloads makes them attractive platforms for personalised medicine and precision drug delivery. Increasing understanding of disease-specific molecular targets has encouraged the development of ligand-directed liposomes capable of selectively accumulating within diseased tissues while sparing healthy organs, thereby improving therapeutic selectivity⁷

Despite remarkable progress, several scientific and technological challenges continue to limit the widespread application of liposomal drug delivery. Issues including physical instability, drug leakage, oxidation of phospholipids, limited encapsulation efficiency, complex manufacturing processes and regulatory standardisation remain areas of active investigation. Considerable progress was made during 1970s and 1980s in the field of liposome stability leading to long circulation times of liposomes after intravenous administration resulting in the improvement in bio-distribution of liposome. The important anti-tumour drug doxorubicin had been formulated as liposome in 1980s to improve the therapeutic index. Liposome research is gaining importance in biological, pharmaceutical and medical research because liposomes seem to be the most effective carriers for the introduction of all kinds of agents into cells.⁸

These difficulties can be minimized by using temperature sensitive liposomes. These liposomes were developed by Yatvin et al & Weinstein et al. and others for better orientation to targeted sites.⁹

These liposomes are formed by a mixture of synthetic lipids such as dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC), that exhibits a gel to sol transition at temperature few degrees above the physiological temperature, a range easily obtainable by local hyperthermia. It was postulated that such liposomes would remain reasonably stable in the vascular system at the normal body temperature (37°C), and when passing through the heated area they would release their contents when the transition temperature is attained.^{10, 11, 12}

History of Liposomes

The history of liposomes represents one of the most significant milestones in pharmaceutical nanotechnology. The concept emerged during investigations into biological membrane organisation rather than drug delivery. (Deamer DW et al, 2010)

Over time, fundamental discoveries regarding phospholipid self-assembly transformed liposomes into one of the most successful nanoscale drug delivery systems ever developed¹³

When dispersed in aqueous environments, these molecules spontaneously arrange themselves into bilayer structures to minimise free energy. Although

this self-assembly phenomenon was recognised, its pharmaceutical significance was not immediately appreciated^{14,62}

A major breakthrough occurred in **1965**, when the British haematologist **Dr Alec D. Bangham** and his colleagues at the Babraham Institute in Cambridge accidentally observed microscopic phospholipid vesicles while examining phospholipid dispersions using electron microscopy. This discovery fundamentally changed scientific understanding of membrane biology and initiated decades of research into phospholipid-based delivery systems¹³

During the late 1960s, liposomes were primarily employed as experimental membrane models rather than therapeutic carriers. Liposomes rapidly became indispensable tools in cell biology, biochemistry and membrane biophysics¹⁵

The pharmaceutical potential of liposomes was recognised in the early 1970s when researchers demonstrated that biologically active compounds could be encapsulated within phospholipid vesicles. Among the pioneering scientists who advanced this concept was **Gregoriadis**, who demonstrated that liposomes could deliver enzymes and other therapeutic molecules to specific organs, laying the foundation for targeted drug delivery¹⁴

Throughout the 1980s, advances in lipid chemistry and formulation technology substantially improved liposome stability and encapsulation efficiency. Cholesterol incorporation into phospholipid bilayers enhanced membrane rigidity and reduced drug leakage. Remote loading techniques further improved the encapsulation of weakly basic drugs, allowing significantly higher drug loading efficiencies than earlier passive loading methods¹⁶

Another landmark achievement occurred during the early 1990s with the development of polyethylene glycol (PEG)-coated or "stealth" liposomes. Surface modification with PEG dramatically reduced recognition by the mononuclear phagocyte system, thereby prolonging circulation time and increasing drug accumulation within tumour tissues via the enhanced permeability and retention (EPR) effect. The first regulatory approval of a liposomal drug represented a major milestone for nanomedicine. Liposomal formulations of amphotericin B and doxorubicin demonstrated that nanotechnology could significantly reduce toxicity while maintaining therapeutic effectiveness. Since then, numerous liposomal products have entered clinical practice for the treatment of fungal infections, cancers, pain management and inflammatory disorders¹⁷ Their remarkable adaptability, favourable safety profile and proven clinical success continue to drive innovation across pharmaceutical sciences, biotechnology and regenerative medicine⁵

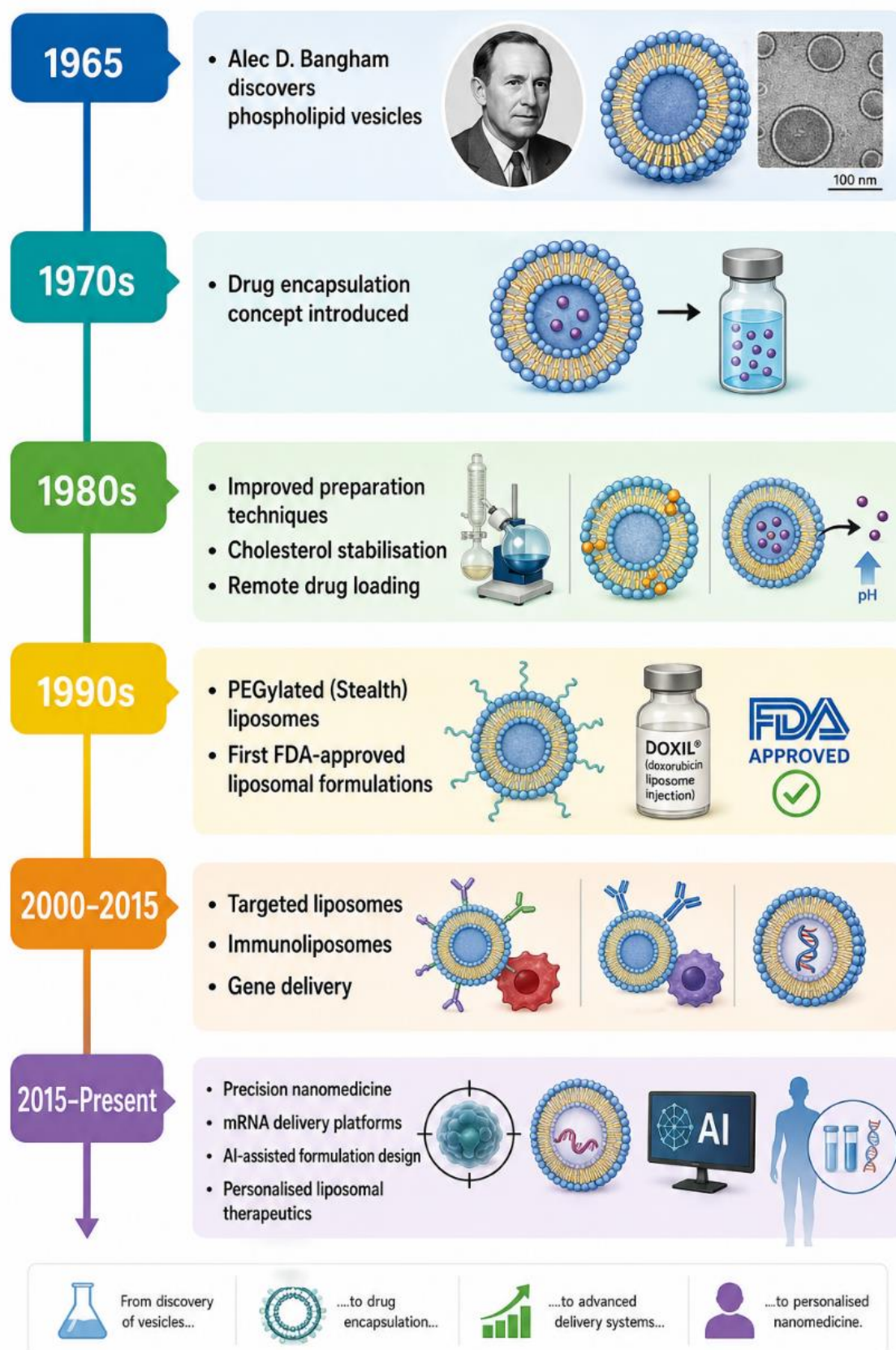


Figure 1. Historical Timeline of Liposome Development (Suggested Figure)

Structure and Composition of Liposomes

Liposomes are spherical vesicular systems composed primarily of one or more concentric phospholipid bilayers enclosing an aqueous core. Unlike conventional

drug carriers, liposomes possess both hydrophilic and hydrophobic domains, enabling them to simultaneously encapsulate water-soluble compounds within the aqueous compartment and lipid-soluble molecules

within the phospholipid bilayer. This dual drug-loading capability distinguishes liposomes from many other nanocarriers and underpins their broad pharmaceutical applicability³

The self-assembly of phospholipids into bilayer vesicles is driven by the amphiphilic nature of phospholipid molecules. Each phospholipid molecule contains a polar hydrophilic head group, usually comprising phosphate and choline, ethanolamine, serine or glycerol, together with two non-polar hydrophobic fatty acid chains. This spontaneous arrangement minimises the system's free energy and results in the formation of stable bilayer membranes¹⁸

In addition to phospholipids, cholesterol is incorporated into many liposomal formulations to improve membrane stability and mechanical strength. Cholesterol intercalates between phospholipid molecules within the bilayer, reducing membrane permeability, preventing phospholipid crystallisation and minimising drug leakage. The amount of cholesterol incorporated influences membrane rigidity, drug retention and circulation behaviour. Appropriate cholesterol concentrations therefore contribute significantly to the pharmaceutical performance of liposomal formulations¹⁷

Modern liposomes frequently include additional lipid derivatives designed to improve therapeutic performance. Polyethylene glycol (PEG)-conjugated phospholipids provide a hydrophilic steric barrier around the liposome surface, reducing protein adsorption and delaying clearance by the mononuclear phagocyte system. Such PEGylated liposomes exhibit prolonged circulation times and enhanced passive accumulation in tumour tissues through the enhanced permeability and retention (EPR) effect. Other functional lipids incorporate targeting ligands, antibodies, peptides or carbohydrates to facilitate active targeting towards specific cell types or disease biomarkers¹

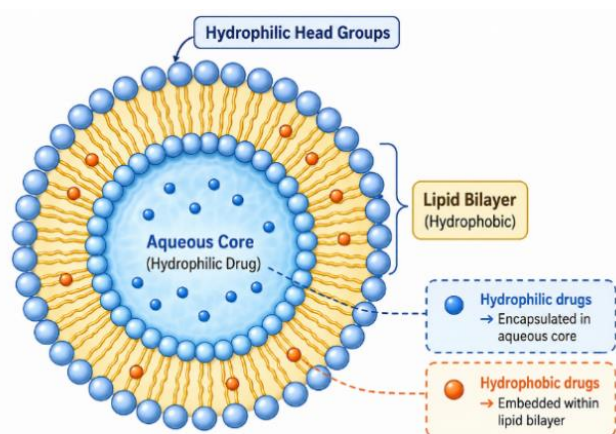


Figure 2. Basic Structure of a Liposome (Suggested Figure)

CLASSIFICATION OF LIPOSOMES

Liposomes may be classified according to several criteria, including vesicle size, number of phospholipid bilayers, preparation technique, surface characteristics and functional modifications. Such classification facilitates the selection of appropriate formulations for specific therapeutic applications and provides insight into their pharmacokinetic behaviour¹⁹

✚ **Small unilamellar vesicles (SUVs)** contain a single phospholipid bilayer and generally measure between 20 and 100 nm in diameter. Owing to their small size, SUVs exhibit enhanced tissue penetration, prolonged circulation and efficient intracellular uptake, making them particularly suitable for anticancer drug delivery and gene therapy applications¹⁸

✚ **Large unilamellar vesicles (LUVs)** also possess a single phospholipid bilayer but are considerably larger, typically ranging from 100 nm to 1 µm. Their larger internal aqueous volume permits encapsulation of greater quantities of hydrophilic drugs, proteins and nucleic acids. Consequently, LUVs are widely utilised in vaccine delivery, enzyme replacement therapy and protein-based therapeutics³

✚ **Giant unilamellar vesicles (GUVs)** exceed one micrometre in diameter and closely resemble biological cells in size. Although they are rarely employed for routine pharmaceutical drug delivery, GUVs are extensively used as experimental models in membrane biophysics, synthetic biology and cellular interaction studies. Their large dimensions facilitate direct microscopic observation of membrane dynamics and lipid-protein interactions⁵

✚ **Multilamellar vesicles (MLVs)** comprise multiple concentric phospholipid bilayers resembling the layers of an onion. These vesicles are typically larger than 500 nm and contain several aqueous compartments separated by phospholipid membranes. MLVs are relatively simple to prepare and exhibit excellent physical stability; however, their large size often limits tissue penetration and intravenous administration. Nevertheless, they remain valuable for topical formulations, depot drug delivery and sustained-release applications⁴

✚ **Stealth liposomes**, also known as PEGylated liposomes, possess polyethylene glycol molecules attached to their surface. The hydrophilic PEG layer reduces protein adsorption and decreases recognition by macrophages, substantially prolonging systemic circulation. These formulations have become particularly important in cancer chemotherapy owing to their ability to exploit the enhanced permeability and retention effect observed in many solid tumours¹⁷

✚ **Targeted liposomes**, frequently referred to as immunoliposomes, incorporate antibodies, peptides, aptamers or other targeting ligands onto their surface. These ligands selectively bind receptors overexpressed on diseased cells, thereby facilitating receptor-mediated endocytosis and improving drug accumulation within target tissues while minimising systemic toxicity⁷

Physicochemical Properties of Liposomes: The therapeutic performance of liposomes depends largely upon their physicochemical properties, which govern drug encapsulation, circulation time, stability and interaction with biological systems. Important parameters include vesicle size, lamellarity, surface charge, membrane fluidity, lipid composition, encapsulation efficiency, polydispersity index and zeta potential³

Negative surface charges may improve colloidal stability through electrostatic repulsion but can also alter biodistribution and immune recognition²⁰

Mechanism of Liposome Formation

Liposome formation is fundamentally based on the spontaneous self-assembly of amphiphilic phospholipid molecules in aqueous environments. Upon hydration, phospholipids arrange themselves into bilayers in which hydrophobic fatty acid chains face inward, shielded from water, while hydrophilic head groups remain exposed to the surrounding aqueous medium. Continued hydration results in the closure of these bilayers into spherical vesicles, thereby eliminating energetically unfavourable exposed membrane edges¹³

The final characteristics of the resulting liposomes are influenced by several formulation variables, including lipid composition, hydration conditions, temperature, ionic strength, mechanical agitation and preparation technique. Processes such as sonication, extrusion, homogenisation and microfluidisation enable researchers to control vesicle size and lamellarity, thereby tailoring liposomal properties for specific pharmaceutical applications¹⁸

The spontaneous nature of liposome formation, combined with advances in lipid chemistry and pharmaceutical engineering, has enabled the development of increasingly sophisticated nanocarriers capable of delivering a broad spectrum of therapeutic agents with high efficiency and safety⁴

Methods of Preparation of Liposomes

The therapeutic performance of liposomal drug delivery systems is strongly influenced by the method used for their preparation. The selected preparation technique determines vesicle size, lamellarity, encapsulation efficiency, drug retention, stability and scalability.²⁷

An ideal preparation method should produce liposomes with uniform particle size, high drug loading, minimal lipid degradation, reproducible quality and suitability for large-scale pharmaceutical manufacturing.

Over the past five decades, numerous preparation techniques have been developed to overcome the limitations associated with conventional liposome production⁵

Thin-Film Hydration Method (Bangham Method)

The thin-film hydration method, originally developed by **Bangham and colleagues**, remains the most widely used laboratory technique for preparing liposomes.³⁴

In this method, phospholipids and cholesterol are dissolved in an organic solvent such as chloroform or methanol.³¹

The solvent is then removed under reduced pressure using a rotary evaporator, leaving a thin lipid film deposited on the inner surface of a round-bottom flask.³²

Subsequent hydration of the lipid film with an aqueous buffer at a temperature above the lipid phase transition temperature results in spontaneous formation of multilamellar vesicles (MLVs)¹³

Although this method is simple, inexpensive and highly versatile, it generally produces heterogeneous liposome populations with broad particle size distributions.³³

Additional size-reduction techniques such as sonication or membrane extrusion are therefore required to obtain small unilamellar vesicles suitable for intravenous administration. Despite these limitations, the Bangham method remains the foundation of many experimental liposomal formulations due to its simplicity and flexibility¹⁸

Reverse-Phase Evaporation Method

The reverse-phase evaporation method was developed to improve the encapsulation efficiency of hydrophilic drugs. In this technique, phospholipids are dissolved in an organic solvent, while the aqueous drug solution is emulsified into the organic phase using sonication or homogenisation. Removal of the organic solvent under reduced pressure causes gradual formation of lipid bilayers surrounding aqueous droplets, eventually producing large unilamellar vesicles with relatively high aqueous drug entrapment¹⁵

Compared with thin-film hydration, reverse-phase evaporation generally yields higher encapsulation efficiencies for proteins, peptides and water-soluble therapeutic agents. However, prolonged exposure of sensitive biomolecules to organic solvents may reduce biological activity, limiting its application for certain pharmaceuticals³

Ethanol Injection Method

The ethanol injection method represents a relatively simple and rapid approach for preparing small unilamellar liposomes. Lipids are dissolved in ethanol and injected slowly into an aqueous phase under continuous stirring. As ethanol rapidly diffuses into the aqueous medium, phospholipids self-assemble into nanoscale vesicles. The residual ethanol is subsequently removed by evaporation or dialysis⁵

This method avoids the use of highly toxic organic solvents and facilitates continuous manufacturing. Nevertheless, encapsulation efficiency for hydrophilic compounds is generally lower than that achieved using reverse-phase evaporation, and careful control of injection rate and mixing conditions is required to ensure uniform particle size²⁰

Detergent Removal Method

The detergent removal method is particularly useful for incorporating membrane proteins into liposomal bilayers. Phospholipids are initially solubilised using detergents to form mixed micelles. Gradual removal of the detergent by dialysis, gel filtration or adsorption onto hydrophobic resins promotes spontaneous liposome formation. This technique preserves the structural integrity of membrane proteins and has become valuable in membrane biology and vaccine development¹⁹

Sonication Method

Sonication is commonly employed to reduce the size of multilamellar vesicles produced by thin-film hydration. High-frequency ultrasonic energy disrupts larger vesicles into smaller unilamellar liposomes. Probe sonicators generally produce smaller particles than bath sonicators but may generate localised heating and titanium particle contamination if excessive processing occurs¹⁸

Although sonication effectively reduces vesicle size, prolonged exposure to ultrasonic energy may degrade phospholipids and destabilise temperature-sensitive drugs. Consequently, optimisation of sonication time and cooling conditions is essential for preserving formulation quality⁴

French Press Extrusion

Dispersions of multilamellar vesicles can be reduced in size by extrusion at high pressures through a French press. This method is originally ascribed to Hamilton & Goerke, although no details were reported. A similar method has been documented in greater detail by Barenholz and colleagues²⁴

Dispersions of lipids are placed in the French press and extruded at 20,000 lbs/in² at 4°C. Dispersions extruded only once consist of a heterogeneous collection of vesicles, including MLV, with approximately 60% of the vesicles occurring in the 250-500 Å size range. Multiple extrusion of the preparation resulted in a progressive decrease in the size heterogeneity, and after four extrusions 94% of the vesicles ranged in size from 315-525 Å in diameter. The resulting vesicles are somewhat larger than sonicated unilamellar vesicles and the technique should be applicable to a wide variety of lipid compositions²³

It appears to be a simple, reproducible, and nondestructive technique that makes it possible to prepare large volumes of vesicles at high lipid concentrations with a minimal dilution. Thus the encapsulation of the aqueous phase should be relatively efficient, and this preparation should be a valuable tool to complement other methods for forming small unilamellar vesicles.²³

Extrusion:-

Step 1. Preparation of Thin Lipid Film

The required amounts of phospholipids and cholesterol are dissolved in chloroform or a chloroform-methanol mixture in a round-bottom flask. The solvent is removed using a rotary evaporator under reduced pressure to produce a thin, uniform lipid film on the flask wall. Residual solvent is eliminated by drying under vacuum for several hours.

Step 2. Hydration of Lipid Film

The dried lipid film is hydrated using an aqueous buffer maintained above the lipid phase transition temperature. Gentle shaking or vortexing disperses the lipids and forms **multilamellar vesicles (MLVs)**. Hydration usually continues for 30–60 minutes to ensure complete swelling of the lipid film.

Step 3. Freeze-Thaw Cycles (Optional)

The liposomal suspension may undergo 5–10 freeze-thaw cycles by alternating between liquid nitrogen and warm water (approximately 50–60°C). This process enhances encapsulation efficiency and promotes equilibration of aqueous compartments.

Step 4. Extrusion

The MLV suspension is transferred into an extruder fitted with polycarbonate membranes.

The extrusion temperature should be maintained **5–10°C above the phospholipid transition temperature**.

The suspension is repeatedly passed through membranes of decreasing pore size.

Typical sequence:

- 400 nm membrane (5–10 passes)
- 200 nm membrane (5–10 passes)
- 100 nm membrane (10–15 passes)

Many researchers perform **10–21 extrusion cycles** through the final membrane to obtain a narrow particle-size distribution.

Repeated extrusion converts multilamellar vesicles into large unilamellar vesicles with low polydispersity.

Step 5. Characterization

After extrusion, liposomes are characterized for:

- Particle size (Dynamic Light Scattering)
- Polydispersity Index (PDI)
- Zeta potential
- Encapsulation efficiency
- Lamellarity (Cryo-TEM or electron microscopy)
- Stability studies²⁵

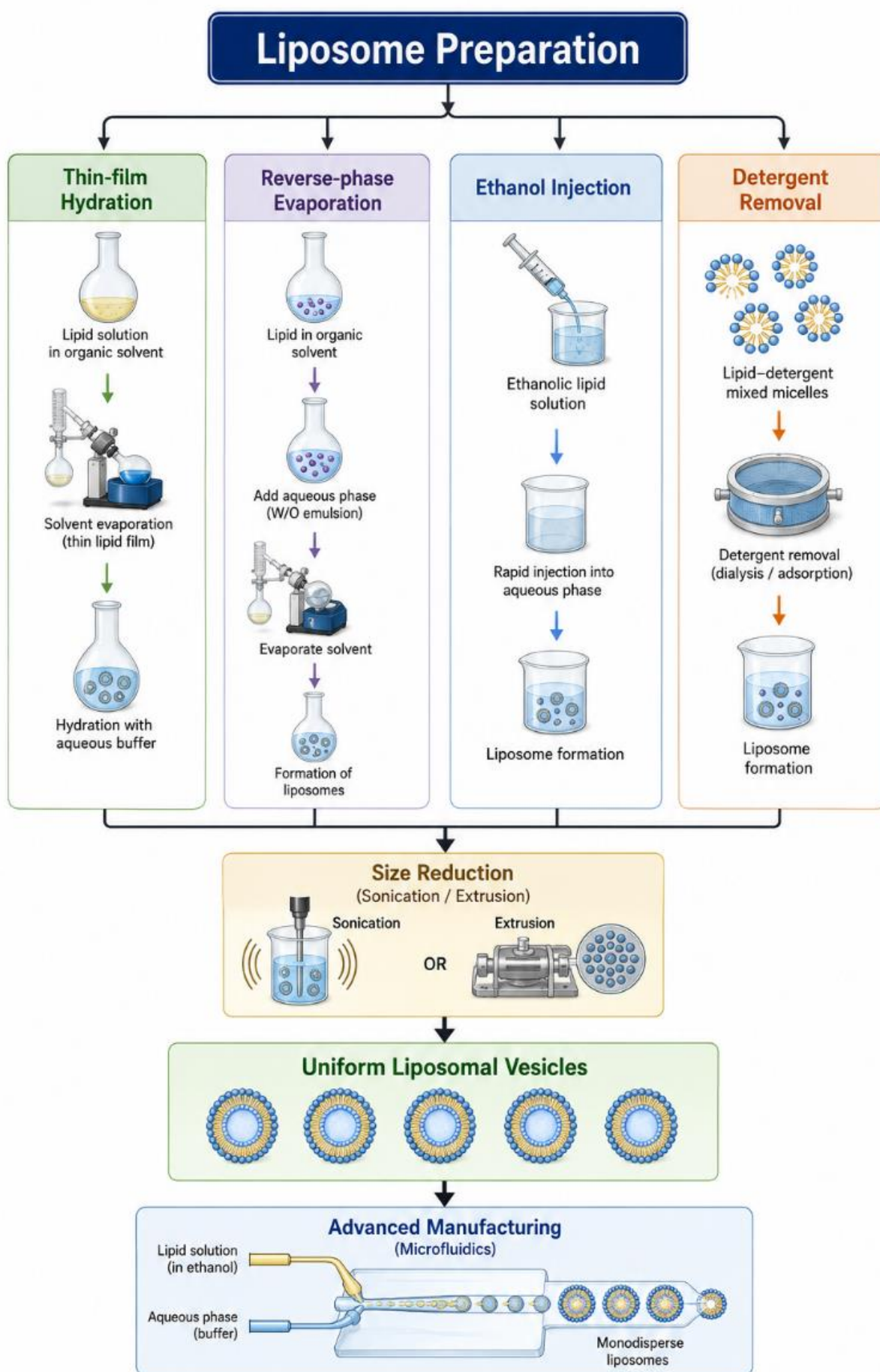


Figure 3. Common Methods for Liposome Preparation (Suggested Figure)

Supercritical Anti-Solvent (SAS) Method

In this method, a solution containing an organic solvent and the solute (lipids and active drugs) is placed in contact with a SCF, (such as SC-CO₂) which is (completely) miscible with the organic solvent but acts as anti-solvent for the solute.³⁵

The dissolution of SC-CO₂ in the liquid phase and the successive organic solvent extraction favors the precipitation of the lipidic nanoparticles. The processed solution is successively hydrated in an aqueous buffer solution, leading to the formation of liposomes. To remove any organic solvent in excess, a washing stage (with pure CO₂) is finally performed.

The versatility of this method may stimulate the industrial applications in a large variety of technology fields, such as pharmaceuticals, cosmetics, textiles, and nutraceuticals.

By using the SuperLip process, lutein (a lipophilic ophthalmic nano-drug) has been encapsulated in liposomes with mean diameters between 153 ± 38 and 267 ± 56 nm, with high lutein encapsulation efficiencies (between $86.5 \pm 0.4\%$ and $97.8 \pm 1.2\%$)³⁷

The variation of temperature for the production of liposomes showed a significant impact on lutein retention time (within the double lipid layer). Lutein drug release from liposomes produced at 35 °C ended after almost 4.5 days, whereas liposomes produced at 40 °C showed a faster lutein release in 3 days. Then, vesicles obtained at 45 °C released their lutein content in only 2 days³⁷

This combined approach allowed for obtaining a meta-carrier (i.e., a carrier within another carrier) with a drug release that works with two mass-transfer resistances (in series). The first mass resistance was given by the structure of the aerogel, during the release of ampicillin not entrapped into liposomes.³⁹

The second mass resistance was given by the lipidic double layer of liposomes. The initial liposomes (with a diameter of 200 ± 77 nm and EE of $69.5 \pm 1.2\%$) were successfully entrapped into the aerogels (as confirmed by EDX analysis), while the antibiotic entrapped into simple liposomes alone was totally released after about 3 days. The antibiotic entrapped into a meta-carrier reached a plateau after about 5 days³⁷

Drug Loading Techniques

Efficient drug loading is a critical determinant of liposomal therapeutic performance. The amount of drug encapsulated influences dosage requirements, therapeutic efficacy, manufacturing costs and clinical success. Drug loading strategies are broadly categorised into **passive loading** and **active (remote) loading**, depending on whether drug incorporation occurs during or after liposome formation¹⁷

Passive Loading

Passive loading involves incorporating therapeutic agents during liposome formation. Hydrophilic drugs dissolve in the aqueous hydration medium and become

entrapped within the internal aqueous compartments, whereas hydrophobic drugs partition into the phospholipid bilayer during lipid film formation. Amphiphilic drugs may distribute between both compartments depending on their partition coefficient³

In the passive loading method, drugs **are loaded during the liposomes** preparation. This method can be performed by means of solvent dispersion, mechanical dispersion, and detergent removal methods. For example, in the thin TFH method, the lipophilic drugs are incorporated (in large amounts) within the lipid bilayers during the first stages of the liposomes' self-assembly process.³¹

Active (Remote) Loading

The main aim of the liposome formation process is to create nanoformulations dedicated to the efficient transport and delivery of lipophilic (within the lipid bilayer), hydrophilic (in the aqueous core), and amphiphilic (partitioned at the bilayers surface) drugs. The amount of drug loading depends not only on the method used for liposome preparation, but also on the drugs' chemical characteristics and on the composition and physicochemical properties of the liposomes (such as lipids' concentrations and charge characteristics, drug/lipid ratios).

Active loading has revolutionised liposomal drug delivery by achieving substantially higher encapsulation efficiencies than passive loading. In this approach, pre-formed liposomes containing transmembrane pH or ion gradients actively accumulate weakly basic drugs across the lipid bilayer. Once inside the vesicle, drug molecules become protonated or precipitate, preventing their diffusion back across the membrane¹⁶

Remote loading offers numerous advantages, including encapsulation efficiencies exceeding 90%, excellent drug retention, reduced leakage during storage and enhanced clinical performance. This technology is employed in several commercially approved liposomal medicines, including liposomal doxorubicin¹

Characterisation of Liposomes

Comprehensive physicochemical characterisation is essential to ensure the quality, safety and efficacy of liposomal formulations. Characterisation provides valuable information regarding particle size, morphology, surface charge, encapsulation efficiency, stability and drug release behaviour. Regulatory authorities require rigorous evaluation of these parameters before clinical approval¹⁷

Particle Size Analysis

Particle size is commonly measured using **Dynamic Light Scattering (DLS)**, laser diffraction or nanoparticle tracking analysis. Liposome size influences biodistribution, circulation time, tissue penetration and cellular uptake. Nanometre-sized liposomes generally exhibit superior pharmacokinetic properties for systemic drug delivery³

Zeta Potential

Zeta potential measures surface electrical charge and provides information regarding colloidal stability. High positive or negative zeta potential values generate electrostatic repulsion between particles, reducing aggregation and improving suspension stability. Neutral liposomes generally exhibit lower zeta potential but often demonstrate favourable biocompatibility²⁰

Morphological Examination

Morphological evaluation is performed using:

- Transmission Electron Microscopy (TEM)
- Scanning Electron Microscopy (SEM)
- Cryogenic Electron Microscopy (Cryo-EM)
- Atomic Force Microscopy (AFM)

These techniques reveal vesicle shape, lamellarity, membrane integrity and structural uniformity⁵

Encapsulation Efficiency

Encapsulation efficiency represents the percentage of drug successfully incorporated into liposomes relative to the total amount added during formulation. Free drug is separated using centrifugation, dialysis, gel filtration or ultrafiltration before quantitative analysis by UV spectroscopy, HPLC or LC-MS. High encapsulation efficiency reduces dosage requirements and improves therapeutic effectiveness¹⁷

In Vitro Drug Release Studies

Drug release profiles are commonly evaluated using dialysis bags, Franz diffusion cells or flow-through systems under physiological conditions. These studies determine release kinetics and help predict in vivo therapeutic performance. Controlled release is particularly desirable for anticancer drugs, reducing systemic toxicity while maintaining prolonged therapeutic concentrations⁷

Quality Control Parameters

Quality control is indispensable for ensuring batch-to-batch consistency and regulatory compliance. Critical quality attributes include:

- Particle size and size distribution
- Polydispersity index (PDI)
- Zeta potential
- Drug content
- Encapsulation efficiency
- Sterility
- Endotoxin levels
- Residual organic solvent
- pH
- Osmolality

- Stability under storage conditions

Modern pharmaceutical manufacturers increasingly employ **Quality by Design (QbD)** principles and **Process Analytical Technology (PAT)** to optimise formulation development, manufacturing processes and product consistency. These systematic approaches improve robustness, facilitate regulatory approval and reduce manufacturing variability¹

MECHANISMS OF DRUG RELEASE FROM LIPOSOMES

The therapeutic efficacy of liposomal drug delivery systems is largely determined by the manner in which the encapsulated drug is released after administration. An ideal liposomal formulation should retain its payload during circulation, protect it from premature degradation and release the drug at the target site in a controlled and predictable manner. The release behaviour depends upon several formulation-related factors, including lipid composition, membrane fluidity, vesicle size, surface modification and the physicochemical properties of the encapsulated drug. Environmental factors such as temperature, pH, enzymatic activity and mechanical stress also influence drug release kinetics³

Drug release from liposomes generally occurs through four principal mechanisms: passive diffusion, liposomal degradation, membrane fusion and environmentally triggered release. These mechanisms may occur independently or simultaneously depending on the formulation and biological environment⁴

✚ Passive Diffusion

Passive diffusion is the simplest mechanism of drug release, whereby encapsulated molecules gradually diffuse across the phospholipid bilayer into the surrounding biological fluids. The diffusion rate is influenced by membrane permeability, lipid composition, cholesterol content, drug molecular size and lipophilicity. Small lipophilic molecules generally diffuse more rapidly than large hydrophilic compounds. Increasing membrane rigidity through cholesterol incorporation reduces passive leakage and prolongs drug retention¹⁷

Passive diffusion contributes to sustained drug release, which is particularly advantageous in chronic disease management by maintaining therapeutic concentrations over extended periods and reducing dosing frequency²⁰

✚ Liposomal Degradation

Following systemic administration, liposomes interact with plasma proteins, enzymes and immune cells. Phospholipases gradually hydrolyse phospholipid molecules, leading to destabilisation of the bilayer and subsequent release of encapsulated drugs. In addition, macrophages of the mononuclear phagocyte system internalise liposomes through phagocytosis. Intracellular lysosomal enzymes degrade the lipid bilayer, resulting in intracellular drug release¹⁹

This mechanism is particularly valuable when therapeutic agents are intended to accumulate within

macrophage-rich tissues such as the liver, spleen and bone marrow, as in the treatment of certain infectious diseases and haematological malignancies ¹

✚ Membrane Fusion

Liposomes may directly fuse with cellular plasma membranes owing to the structural similarity between phospholipid bilayers. Membrane fusion allows direct transfer of encapsulated molecules into the cytoplasm without complete liposome degradation. This process enhances intracellular drug delivery and is particularly important for nucleic acids, proteins and gene-editing systems that require cytoplasmic or nuclear access ³

✚ Stimuli-Responsive Drug Release

Recent advances in nanotechnology have enabled the development of smart liposomes capable of releasing their payload selectively in response to specific environmental stimuli. These formulations improve therapeutic precision while minimising systemic toxicity ^{4, 63}

✚ Temperature-sensitive liposomes

Temperature-sensitive liposomes contain phospholipids with phase transition temperatures slightly above physiological temperature. Localised hyperthermia (approximately 40–42°C) increases membrane permeability, resulting in rapid drug release at heated tumour sites. This strategy has shown considerable promise in cancer chemotherapy ²¹

✚ pH-sensitive liposomes

Many pathological tissues, including solid tumours and inflamed regions, exhibit acidic microenvironments. pH-sensitive liposomes remain stable under physiological conditions but destabilise under acidic conditions, promoting selective drug release within diseased tissues ⁷

✚ Enzyme-responsive liposomes

Certain diseases are associated with elevated concentrations of specific enzymes such as phospholipases, matrix metalloproteinases and proteases. Enzyme-responsive liposomes undergo structural degradation upon exposure to these enzymes, enabling site-specific drug release ²²

✚ Ultrasound- and light-responsive liposomes

External physical stimuli including ultrasound, laser irradiation and magnetic fields may also trigger liposomal drug release. These externally controlled systems provide clinicians with temporal and spatial control over drug delivery, representing an important step towards precision medicine ⁴

✚ Pharmacokinetics and Bio-distribution of Liposomes

One of the major advantages of liposomal drug delivery lies in its ability to modify the pharmacokinetic profile of encapsulated therapeutic agents. Liposomal encapsulation influences drug absorption, distribution, metabolism and elimination, thereby improving therapeutic efficacy while reducing systemic toxicity ¹

FACTORS AFFECTING DRUG RELEASE FROM LIPOSOMES :-

Drug release from liposomes is influenced by several formulation, physicochemical, and physiological factors^{40, 41, 42, 43, 44:}

✚ Lipid composition

- Type of phospholipid used.
- Saturated vs. unsaturated lipids.
- Phospholipid-to-cholesterol ratio.
- More rigid lipid bilayers generally slow drug release.

✚ Cholesterol content

- Cholesterol increases membrane rigidity and reduces permeability.
- Higher cholesterol concentration generally decreases drug leakage and slows release.

✚ Phase transition temperature (T_c)

- Lipids with higher T_c form more rigid membranes at body temperature.
- This generally results in slower drug release.

✚ Drug-lipid interaction

- Strong interaction between the drug and lipid bilayer can retard release.
- Drug location within the liposome also matters:

- Hydrophilic drugs → aqueous core
- Lipophilic drugs → lipid bilayer

✚ Particle size

- Smaller liposomes have a larger surface-area-to-volume ratio and may show faster drug release.
- Larger, more rigid vesicles may release drug more slowly.

✚ Surface charge

- Cationic, anionic, or neutral lipids influence membrane stability and interaction with biological components.
- Surface charge can therefore affect drug leakage and release⁵¹.

✚ Bilayer permeability

- Membrane permeability determines how easily the drug can diffuse through the lipid bilayer.
- Greater permeability → faster release.

✚ Drug concentration/loading

- Higher drug loading may increase the concentration gradient and promote faster release.
- Excess drug may also destabilize the vesicle.

✚pH of the medium

a) Changes in pH can alter drug ionization and its partitioning between the aqueous phase and lipid membrane.

b) This can significantly affect release.⁵²

✚Temperature

a) Increasing temperature can increase lipid mobility and membrane permeability, resulting in faster drug release.

✚Ionic strength and composition of release medium

a) Salts and ions can affect vesicle stability, aggregation, and membrane permeability.

✚Vesicle structure

a) Unilamellar and multilamellar liposomes exhibit different release characteristics.

b) Multilamellar vesicles often provide more prolonged release⁵³.

✚Surface modification

a) PEGylation and other surface modifications can alter membrane stability and interaction with the surrounding medium.

✚Storage conditions

a) Temperature, light, oxidation, and hydrolysis of phospholipids can destabilize liposomes and increase drug leakage.

ADVANTAGES AND DISADVANTAGES OF LIPOSOMES

Table 1. Advantages and Limitations of Liposomal Drug Delivery Systems

Advantages	Limitations
Excellent biocompatibility and biodegradability	High manufacturing cost
Encapsulates both hydrophilic and hydrophobic drugs	Drug leakage during storage
Protects drugs from degradation	Limited long-term physical stability
Controlled and sustained drug release	Phospholipid oxidation and hydrolysis
Reduced systemic toxicity	Complex large-scale manufacturing
Improved pharmacokinetics	Sterilisation challenges
Targeted drug delivery	Batch-to-batch variability
Suitable for gene and vaccine delivery	Complex regulatory approval
Surface modification possible (PEG, ligands)	Cold-chain requirements for some formulations
Clinically proven technology	Relatively short shelf life for certain products

THERAPEUTIC APPLICATIONS OF LIPOSOMES

Liposomes have become one of the most versatile drug delivery platforms in modern medicine. Their ability to enhance drug stability, improve pharmacokinetics and reduce toxicity has led to widespread application across numerous therapeutic areas, including oncology, infectious diseases, vaccination, ophthalmology, dermatology, neurology and regenerative medicine^{4, 54}

Cancer Therapy

Cancer treatment represents the most successful clinical application of liposomal drug delivery. Conventional chemotherapy is often associated with severe systemic toxicity owing to non-selective distribution of cytotoxic agents.²⁸

Liposomes in Infectious Disease Therapy

The treatment of infectious diseases often requires prolonged administration of antimicrobial agents, which may be associated with dose-dependent toxicity, poor tissue penetration and the emergence of antimicrobial resistance. Liposomal drug delivery offers significant advantages by improving drug localisation at infection sites, prolonging circulation time and reducing systemic adverse effects¹⁹

Liposomes in Vaccine Delivery

Vaccination represents one of the most rapidly expanding applications of lipid-based nanocarriers. Liposomes function not only as delivery vehicles but also as immunological adjuvants capable of enhancing both humoral and cellular immune responses. Their phospholipid composition facilitates efficient uptake by antigen-presenting cells, resulting in improved antigen processing and activation of adaptive immunity¹⁹

Liposomal vaccines offer several advantages over conventional vaccine formulations. Encapsulation protects antigens from enzymatic degradation, prolongs antigen persistence at the injection site and enables sustained antigen presentation. Furthermore, liposomes may simultaneously deliver antigens together with immunostimulatory molecules, enhancing vaccine efficacy^{7, 56}

Liposomes in Gene Therapy

Gene therapy aims to treat disease by introducing, replacing or silencing genetic material within target cells. Safe and efficient delivery of nucleic acids remains one of the principal challenges limiting the widespread clinical application of gene therapy. Liposomes have emerged as attractive non-viral vectors because they exhibit lower immunogenicity and improved safety compared with viral delivery systems^{3, 58, 60}

Liposomes in Ophthalmic Drug Delivery

Ocular drug delivery presents unique challenges owing to rapid tear turnover, blinking, nasolacrimal drainage and the presence of multiple anatomical barriers. Conventional eye drops typically exhibit poor ocular bioavailability, with only a small proportion of the administered dose reaching intraocular tissues³

Liposomes in Dermatology and Cosmetic Medicine

The skin represents an attractive route for localised drug delivery; however, the stratum corneum acts as a major barrier limiting drug penetration. Liposomes enhance dermal and transdermal delivery by interacting with skin lipids and facilitating transport across the epidermal barrier⁵

Liposomes in Neurological Disorders

The blood-brain barrier (BBB) represents one of the greatest obstacles to effective treatment of neurological diseases. Its highly selective permeability restricts the

entry of many therapeutic agents into the central nervous system. Liposomes have demonstrated considerable potential for improving drug transport across the BBB through both passive and receptor-mediated mechanisms⁷

Liposomes in Cardiovascular Diseases

Cardiovascular diseases remain the leading cause of mortality worldwide, highlighting the need for improved therapeutic strategies. Liposomal drug delivery offers opportunities to enhance treatment efficacy while reducing systemic toxicity associated with cardiovascular medications⁵

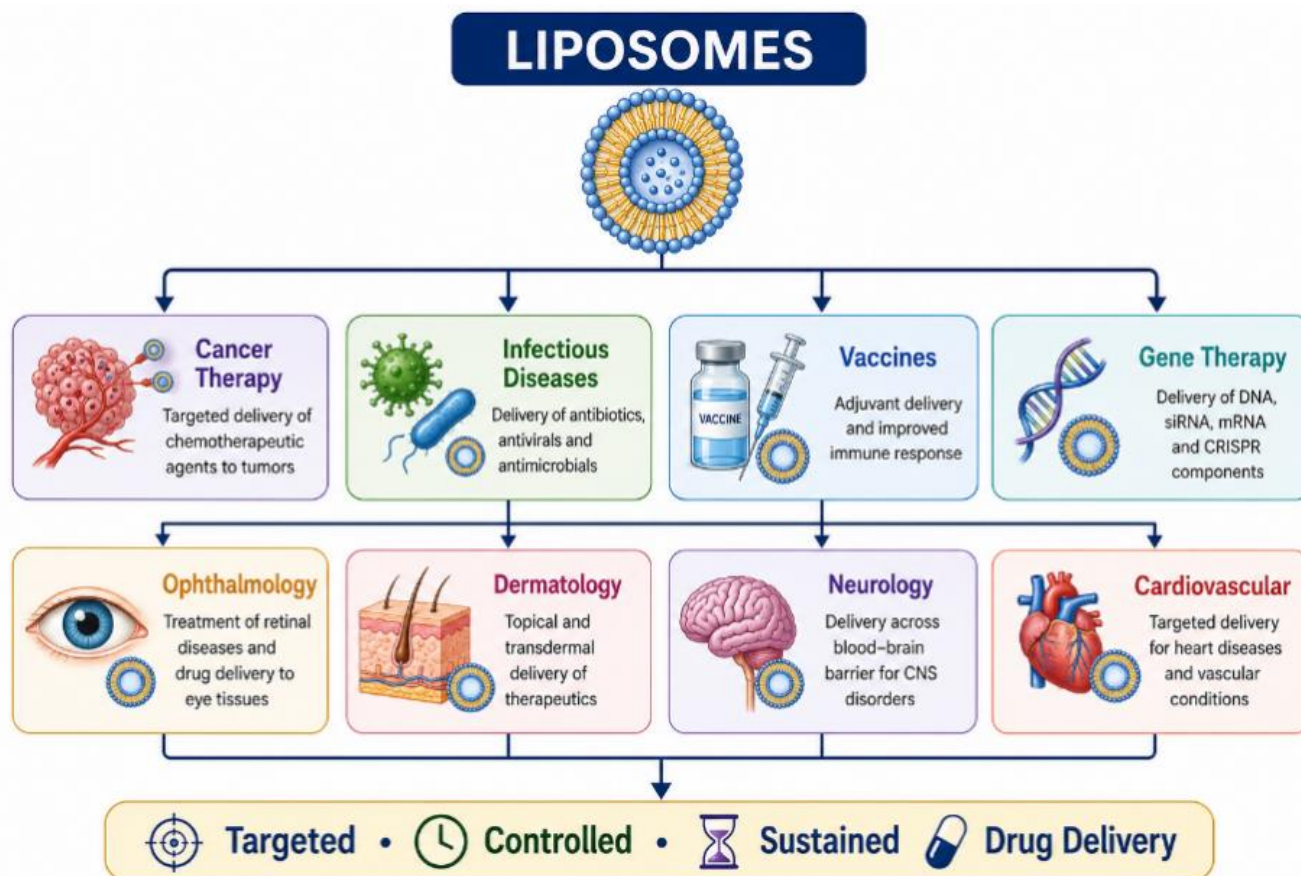


Figure 4. Therapeutic Applications of Liposomes

Liposomes in Pulmonary Drug Delivery

Pulmonary administration offers a non-invasive route for both local and systemic drug delivery due to the lungs' extensive surface area and rich vascularisation. Liposomal formulations designed for inhalation enhance drug retention within pulmonary tissues and reduce systemic toxicity. Encapsulation protects therapeutic agents from enzymatic degradation and provides prolonged drug release in the respiratory tract⁴

Liposomes in Regenerative Medicine

Emerging evidence suggests that liposomes may play an important role in regenerative medicine by delivering growth factors, stem-cell signalling molecules, messenger RNA and gene-editing tools to damaged

tissues. Controlled delivery of these biomolecules enhances tissue repair while reducing unwanted systemic effects³

Future Perspectives

The future of liposomal drug delivery is expected to be driven by advances in nanotechnology, molecular biology, pharmaceutical engineering and computational sciences. Although liposomes have already demonstrated significant clinical success, continuous innovation is expanding their capabilities beyond conventional drug delivery towards precision medicine, personalised therapeutics and intelligent nanomedicine⁴

One of the most promising developments is the design of **next-generation targeted liposomes** capable of selectively recognising diseased cells while sparing healthy tissues. Surface conjugation with antibodies, peptides, aptamers and small-molecule ligands enables receptor-mediated drug delivery to tumour cells, inflamed tissues and infected organs. Such targeted systems are expected to improve therapeutic efficacy, reduce systemic toxicity and enhance patient outcomes, particularly in oncology and autoimmune disorders ^{7, 59}

Artificial intelligence (AI) and machine learning are emerging as powerful tools for accelerating liposomal formulation development. AI-driven algorithms can analyse large experimental datasets to predict optimal lipid compositions, encapsulation efficiency, particle size, drug release kinetics and formulation stability. These computational approaches have the potential to reduce research costs, shorten development timelines and improve manufacturing reproducibility. Digital modelling and simulation may also facilitate optimisation of liposomal formulations before laboratory experimentation, supporting the implementation of Quality by Design (QbD) principles in pharmaceutical manufacturing ^{20, 60}

Future research is also focusing on **multifunctional theranostic liposomes**, which combine therapeutic and diagnostic functions within a single nanocarrier. These systems simultaneously deliver drugs and imaging agents, allowing clinicians to monitor drug distribution, treatment response and disease progression in real time using imaging modalities such as magnetic resonance imaging (MRI), positron emission tomography (PET), computed tomography (CT) and fluorescence imaging. Theranostic liposomes are anticipated to play a central role in personalised oncology and image-guided therapy ^{3, 60, 61}

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