WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Comprehensive Review on Liposome; A Novel Drug Delivery System

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ABSTRACT:

Liposomes, spherical vesicles composed of phospholipid bilayers, have emerged as versatile and promising Nano carriers for drug delivery. This review provides a comprehensive analysis of recent advancements, challenges, and potential applications of liposome-based drug delivery systems. It explores their structural characteristics, formulation techniques, advantages, and limitations, alongside a discussion on scalable production methods and industrial applicability. The ability of liposomes to encapsulate both hydrophilic and lipophilic drugs makes them suitable for targeted therapy, enhancing therapeutic efficacy while minimizing toxicity. Key highlights include clinical applications, recent breakthroughs, and regulatory perspectives based on FDA and EMEA guidelines. This paper also reviews the use of modified liposomes, including long-circulating and surface-modified variants, in clinical trials for cancer treatment and other diseases, underscoring their significance as a novel drug delivery platform.

Keywords: Liposomes, Drug Delivery System, Phospholipids, Nanoparticles, Controlled Release, Targeted Therapy.

1. INTRODUCTION :

Liposomes are self-assembled phospholipid-based vesicles that have garnered significant attention as versatile drug delivery carriers. First described by Alec D. Bangham and colleagues in 1964, these nanostructures can form either unilamellar or multilamellar bilayers, encapsulating both hydrophilic and lipophilic drugs. Their unique structural properties enhanced drug stability, prolonged circulation time, controlled release, and targeted delivery, making them highly effective in various therapeutic applications.

Over the decades, liposomal drug delivery systems have been extensively explored for the administration of small molecules, proteins, nucleic acids, and imaging agents. Their biocompatibility and ability to accumulate at diseased sites via passive or active targeting mechanisms provide a significant advantage over conventional drug formulations. The development of second-generation liposomes, such as PEGylated (stealth) liposomes and ligand-modified targeted liposomes, has further improved their therapeutic efficacy and specificity.

Beyond pharmaceuticals, liposomes have expanded into cosmetics, food technology, and vaccine formulations, highlighting their broad applicability. Given the rapid advancements in liposome-based drug delivery, this review aims to provide a comprehensive overview of their design, mechanisms, and emerging trends in biomedical and pharmaceutical research.

In the ever-evolving field of pharmaceutical sciences, the development of efficient and targeted drug delivery systems has become a critical area of research. Among the various Nano carrier systems, liposomes have emerged as one of the most promising and extensively studied drug delivery vehicles. First discovered in the 1960s, liposomes are microscopic vesicles composed of one or more phospholipid bilayers that can encapsulate both hydrophilic and lipophilic drugs, offering versatility in drug delivery applications.

Liposomes are particularly valued for their biocompatibility, biodegradability, and ability to enhance the therapeutic index of drugs by reducing toxicity and improving bioavailability. Their structural similarity to biological membranes allows them to merge with cell membranes, facilitating efficient drug delivery at the target site. This has made liposomal formulations increasingly popular in the treatment of cancer, infections, and various other chronic diseases.

Over the years, advances in liposomal technology have led to the development of specialized liposomes such as stealth liposomes, immunoliposomes, and stimuli-responsive liposomes, further broadening their potential in targeted and controlled drug delivery.

This review aims to provide a comprehensive overview of liposomes, including their structure, methods of preparation, classification, mechanisms of drug release, advantages, limitations, and their current and potential applications in the pharmaceutical field.

2. HISTORY OF LIPOSOMES:-

Liposomes, microscopic vesicles made of lipid bilayers, were first discovered in the early 1960s by Alec D. Bangham and his colleagues at the Babraham Institute in Cambridge while studying phospholipids. They observed that these lipids spontaneously formed closed spherical structures in water, resembling cell membranes. Initially, liposomes were mainly used as model systems for studying biological membranes.

In the 1970s, G. Gregoriadis proposed their potential use in drug delivery, paving the way for extensive research on their ability to encapsulate and transport therapeutic agents. By the 1980s and 1990s, advancements in liposomal formulations led to the development of various drug delivery systems, including the first FDA-approved liposomal drug, Doxil, for cancer treatment. The introduction of polyethylene glycol (PEG)-coated "stealth" liposomes further enhanced their circulation time and therapeutic efficacy.

From the 2000s onward, liposomes have been widely used in medicine, gene therapy, and vaccine development. They played a crucial role in delivering mRNA COVID-19 vaccines

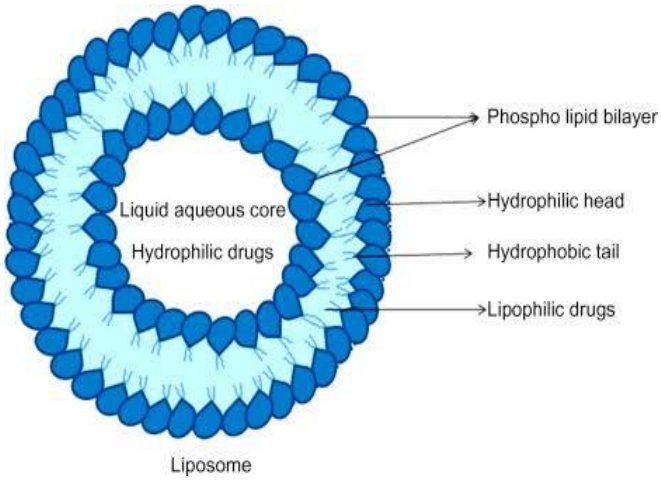

Figure No.1- Liposome

and have been integrated with nanotechnology for targeted and precision medicine

applications. Today, liposomes remain a cornerstone in drug delivery research due to their biocompatibility, biodegradability, and ability to carry both hydrophilic and hydrophobic molecules.

3. MATERIAL AND METHODS :-

STRUCTURE OF LIPOSOME:-

Phospholipids:-

Phospholipids are key components of biological membranes and essential for liposome formation due to their amphiphilic nature. They self-assemble into bilayers, making them ideal for drug delivery by enhancing encapsulation, stability, and controlled release. Classified into glycerophospholipids (e.g., PC, PE, PS, PI, PG) and sphingomyelins, they influence liposome properties such as stability, permeability, and drug release. The phase transition temperature (T^c) affects their behavior, with high-T^c phospholipids like HSPC (53°C) providing increased stability. Additionally, phospholipids regulate surface charge, bio distribution, and clearance, making them crucial for optimizing liposomal formulations.

Cholesterol:-Cholesterol is a crucial component in liposome formation, incorporated at high ratios (up to 2:1 with phosphatidylcholine). As an amphipathic molecule, it inserts into the bilayer with its hydroxyl group facing the aqueous phase and its hydrophobic tail aligned with the lipid acyl chains. Cholesterol enhances liposomal rigidity, mechanical strength, and stability by reducing membrane permeability to hydrophilic compounds. In its absence, liposomes have a more fluid bilayer, leading to destabilization. Thus, cholesterol plays a vital role in maintaining the structural integrity and functionality of liposomal drug carriers.

MECHANISM OF LIPOSOME FORMATION:

Here are the methods for liposome preparation, categorized by technique:

a) Film-hydration method (thin-film method) :-

Process: Lipid-solvent solution evaporated under vacuum to form a thin film, which is hydrated with an aqueous solution to form MLVs (multilamellar vesicles). Particle size reduction can be achieved for SUVs (small unilamellar vesicles).

Applications: Commercial products like Amboise, Visudyne, and Shingrix.

Example: Visudyne - lipids evaporated from dichloromethane, hydrated with lactose, and size reduced by homogenization.

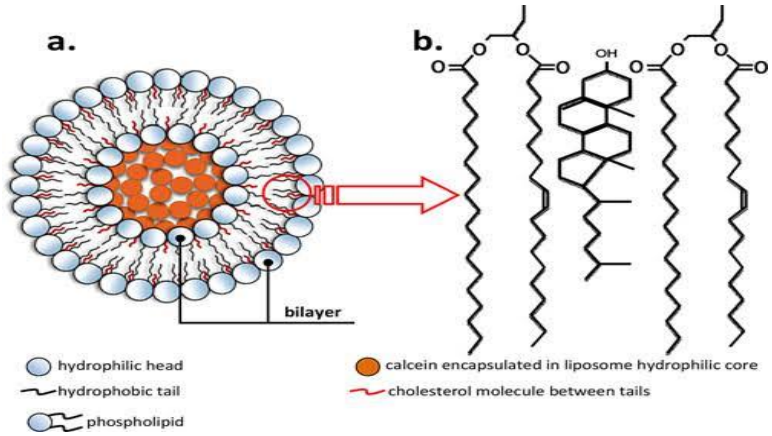

Figure No. 2- Film-hydration method (thin-film method)

b) Double-Emulsification Method:-

Process: Formation of "water-in-oil" emulsion followed by "water-in-oil-in-water" emulsion, solvent removal, and microfiltration.
Applications: DepoCyte, DepoDur, and Expel.
Challenges: Requires aseptic conditions and precise control over lipid concentration and shearing speed.
Disadvantages: Risk of collapse and leakage during solvent removal.

c) Solvent Injection Techniques:-

Process: Lipid and lipophilic substances dissolved in an organic solvent, which is injected into an aqueous buffer to form small unilamellar liposomes.
Advantages: No need for additional energy input for particle size reduction.
Example: Arikayce uses ethanol infusion for amikacin liposome preparation.

d) In Situ Preparation of Liposomes:-

Process: Liposomes are formed just before clinical use by rehydrating a dry lipid cake.
Example: Mepact uses this method for preparation with a dry substance
(MTP-PE, POPC, OOPS) that is hydrated to form liposomes upon clinical use.

e) Size-Reduction Techniques:-

Extrusion: Liposomes are passed through polycarbonate membranes to reduce size.
High-Pressure Homogenization (HPH): MLVs are broken down into smaller vesicles under high pressure.
Applications: Onivyde, Vyxeos, Marqibo, AmBisome, and Visudyne.
Challenges: Size reduction may decrease encapsulation efficiency and alter structure.

f) Drug-Loading Methods:-

Passive Drug-Loading: Drugs encapsulated during liposome formation (low efficiency).
Active Drug-Loading: Drugs actively loaded into preformed liposomes using pH gradients or ionic differences.
Combination Methods: Use of drug-lipid conjugates or hybrid loading techniques to improve encapsulation efficiency.

g) Handshaking Method:-

Process: Lipid film is hydrated and mechanically agitated (e.g., vortexing, shaking) to form liposomes.
Disadvantages: May result in non-uniform vesicle sizes.

h) Sonication Method:-

Process: Ultrasonic waves used to break down liposomes into smaller vesicles (sonication can be either probe or bath).
Challenges: Risk of heating and potential contamination with titanium.

i) Reverse Phase Evaporation Method:-

Process: Formation of inverted micelles via sonication of aqueous and organic phases. This method allows for the encapsulation of high aqueous-to-lipid ratios.

Challenges: High polydispersity index (PDI) and potential for drug stability issues.

j) Detergent Removal Method:-

Process: Mixed micelles formed from lipids and surfactants, followed by detergent removal (e.g., dialysis or chromatography).

Challenges: Hydrophilic drugs may be lost during detergent removal.

k) Dehydration-Rehydration Method:-

Process: Lipids are dispersed in an aqueous solution, followed by sonication and dehydration, then rehydration to form large vesicles.

Challenges: High size heterogeneity of liposomes.

l) Heating Method:-

Process: Lipids are hydrated with an aqueous solution and heated above the phospholipid Tm (transition temperature).

Applications: Suitable for preparing powder inhalable liposomes.

m) pH Jumping Method:-

Process: Phosphatidic acid and phosphatidylcholine exposed to a rapid pH increase, breaking down MLVs into SUVs.

Challenges: The pH ratio influences the vesicle size distribution.

n) Microfluidic Channel Method:+

Process: Lipids dissolved in ethanol or isopropanol are mixed with aqueous solutions in microfluidic channels to form liposomes.

Advantages: High control over size, distribution, and lamellarity.

o) Supercritical Fluidic Method:-

Process: Carbon dioxide (CO2) used to dissolve lipids, followed by a pressure decrease to form liposomes.

Challenges: Expensive, low yield, and special infrastructure requirements.

p) Freeze-Drying (Lyophilization):-

Process: Liposome suspension is frozen and dried to preserve stability, often with cry protectants like sucrose or trehalose.

4. PARAMETERS OF LIPOSOME:

a) **Size and Size Distribution :-**

Affects circulation time, cellular uptake, and bio distribution. Smaller liposomes (<200 nm) typically have longer circulation time.

b) Zeta Potential (Surface Charge):-

Indicates surface charge, affecting stability and interaction with cells. High absolute zeta potential (±30 mV) promotes colloidal stability.

c) Lipid Composition:-

Determines membrane fluidity, permeability, and drug compatibility. Common lipids: phosphatidylcholine (PC), cholesterol, DSPC.

d) Cholesterol Content:-

Modifies membrane rigidity and reduces leakage of encapsulated drug. Usually included to stabilize the bilayer.

e) Encapsulation Efficiency:-

The percentage of drug successfully enclosed within liposomes. Depends on lipid composition, preparation method, and drug properties.

f) Lamellarity:-

Refers to the number of bilayers (unilamellar vs multilamellar). Affects drug loading capacity and release profile.

g) Phase Transition Temperature (Tc):-

Temperature at which lipids shift from solid-like to fluid-like state. Impacts stability, drug release rate, and behavior in vivo.

h) pH Sensitivity:-

Some liposomes are designed to release contents in acidic environments (e.g., tumors). Important for targeted drug delivery.

i) Surface Modification (e.g., PEGylation):-

ii) PEGylation (attaching polyethylene glycol) enhances circulation time and reduces immune recognition. Can be modified with ligands for targeting.

j) Stability (Physical and Chemical):-

Includes shelf-life, resistance to aggregation, and oxidation of lipids. Influenced by storage conditions and composition.

5. DRUG RELEASE STUDIES:

A) Purpose of Drug Release Studies:

To assess the kinetics of drug release from liposomes. To simulate in vivo conditions (pH, temperature, enzymes) for predicting performance. To evaluate stability and control over release rate (e.g., sustained, burst, or triggered release).

B) Key Factors Influencing Drug Release:

C) Common In Vitro Drug Release Methods:

a. Dialysis Bag Method

Liposome suspension is placed in a dialysis bag immersed in a release medium (e.g., PBS). Samples are withdrawn at intervals to measure drug concentration outside the bag.

Advantage: Simple and low-cost.

Limitation: May not accurately mimic in vivo release.

b. Franz Diffusion Cell

Drug release is studied across a membrane between donor and receptor compartments. Common in topical formulations.
c. Ultracentrifugation
At intervals, liposomes are separated from the medium by centrifugation. Supernatant is analyzed for released drug.
d. Gel Filtration/Column Chromatography
Separates free drug from liposome-entrapped drug. Useful for liposomes with slow release.
e. Spectrophotometry/Fluorimetry/HPLC Used to quantify the drug concentration in samples at each time point.

D) Drug Release Kinetics Models:
To understand release mechanisms, data is fitted to various mathematical models:

E) Simulated Conditions:
pH 7.4: Mimics blood/plasma.
pH 5.0–6.0: Mimics endosomal/tumor environment. 37°C: Simulates body temperature. Presence of serum/proteins: Mimics in vivo interactions.

F) Interpretation of Results:
Burst release: Rapid release initially—may occur due to surface-adsorbed drug.
Sustained release: Gradual release over time.
Triggered release: Release occurs in response to a stimulus (pH, temp, enzymes).

G) Applications:
Cancer therapy: Triggered release at tumor site.
Antibiotics: Sustained release to reduce dosing frequency.
Vaccines: Controlled antigen delivery**.**

6. APPLICATION:

A. Ophthalmic Disorders:
Used in treatments for dry eyes, keratitis, corneal transplant rejection, and other eye disorders. Liposome formulations enhance ocular bioavailability, residence time, and reduce drug dosage.
B. Cancer Chemotherapy:
Liposomes serve as carriers for cytotoxic molecules and macromolecules, improving drug delivery to tumors. Enhance drug efficacy by increasing circulation time and targeting cancer cells.
C. Vaccines:
Liposomes act as adjuvants in vaccine delivery, enhancing immune responses. Modify surface with peptides/virus antigens to boost immunity.
D. Gene Delivery:
Cationic liposomes are used for gene transfection (e.g., Lofectamine 2000) and delivery of CRISPR/Cas9 for genetic disorders and cancer treatments.
E. Veterinary Medicine:
Liposomes are utilized for drug delivery in animals.
F. Respiratory Disorders:
Liposomes offer improved sustained release, stability, and reduced toxicity for respiratory treatments via inhalation.
G. Immunological Applications:
Used as immuno adjuvants in vaccine formulations to enhance immune responses.
H. Protein Drug Delivery:
Liposomes enhance drug solubilization and protect protein drugs.
I. Pulmonary Application:
Facilitates pulmonary delivery of drugs due to high solubilization capacity.
J. Cosmetics and Skincare:
Enhances controlled release and penetration of active ingredients like vitamins and antioxidants.
K. Transdermal Drug Delivery:
Applied topically to improve drug delivery through the skin, avoiding first-pass metabolism.
L. Gene Therapy:
Used for delivering genetic material like DNA and RNA, protecting cargo during transport to target cells.
M. Targeted Drug Delivery:
Liposomes can be engineered with ligands for site-specific drug delivery, improving targeting to particular cells or tissues.
N. Protection Against Enzyme Degradation:
Liposomes protect entrapped drugs from enzymatic degradation in circulation.
O. Miscellaneous Applications:
Liposomes are used in biotechnology for drug screening, and as carriers for contrast agents in medical imaging like MRI and ultrasound.
These applications demonstrate the versatility and effectiveness of liposomes across multiple fields, from drug delivery to diagnostics and cosmetics.

7. EVALUATION OF LIPOSOME:-

1.Physical Characterization:

a) Vesicle Shape & Lamellarity: Assessed using electron microscopy and techniques like Freeze Fracture Electron Microscopy and P31 NMR.

- Electron microscopy (e.g., TEM, Cryo-TEM) provides high-resolution images to determine vesicle shape (spherical, multilamellar, unilamellar).
- Freeze Fracture Electron Microscopy gives information on internal lamellae and bilayer organization.
- Phosphorus-31 Nuclear Magnetic Resonance (P31 NMR) helps assess lamellarity and the state of phospholipids.

b) Vesicle Size & Distribution: Determined by various methods like light microscopy, electron microscopy (TEM), laser light scattering, PCS, and atomic force microscopy.Size directly influences drug loading, release kinetics, and biodistribution.

Measured using:

- Light microscopy (for larger vesicles)
- Transmission Electron Microscopy (TEM)
- Dynamic Light Scattering (DLS) / Photon Correlation Spectroscopy (PCS)
- Atomic Force Microscopy (AFM)

c) Microscopic Techniques:

- Optical Microscopy**:** Optical Microscopy is suitable for multilamellar vesicles (>1 µm).
- Cryo-TEM : Cryo-TEM provides a near-native view of liposome size and surface morphology in frozen hydrated state.

d) Diffraction & Scattering Techniques:

Laser Light Scattering (PCS): analyzes Brownian motion of vesicles to determine mean diameter and polydispersity index (PDI).

e) Hydrodynamic Techniques:

Gel Permeation/Ultracentrifuge: Used to separate and estimate vesicle size distribution.

2. Chemical Characterization:

a) Encapsulation Efficiency (EE%):

- Indicates how much drug is successfully entrapped inside the liposome.
- Methods include centrifugation, dialysis, followed by drug quantification using UV-Vis spectroscopy, HPLC, or fluorescence methods.

b) Surface Charge: Electrophoretic light scattering to analyze surface charge and stability.

Influence on lipid-cell interaction, uptake, and delivery efficiency.

c) Surface Hydration: Steric stabilization with hydrophilic coatings to reduce opsonization and improve circulation half-life.

d(Lipid Composition and Purity:

Purpose: Ensure correct lipids are present and free from impurities.

Method:

- Thin Layer Chromatography (TLC)
- High Performance Liquid Chromatography (HPLC)
- Gas Chromatography (GC)

e) pH Measurement:

- Purpose: Determines compatibility with drug and administration route.
- Method: Direct pH meter measurement of liposome suspension.

3. Liposome Structure and Morphology Characterization:

a) TEM & SEM: Used for size, shape, and surface analysis.

b) Dynamic Light Scattering (DLS): For size distribution and polydispersity.

4. Lipid Composition Analysis:

- HPLC: Used to identify and quantify lipids in formulations.
- Thin Layer Chromatography (TLC)
- Purpose: To identify different types of lipids present.
- Principle: Lipids are separated based on polarity on a silica gel plate.
- Gas Chromatography (GC)
- Mass Chromatography

5. Liposome Properties:

a) Encapsulation Efficiency: Measured by UV-Visible or Fluorescence Spectroscopy.

b) Stability: Monitored by observing size, polydispersity, and zeta potential over time.

c) Drug Release Kinetics: In vitro release studies to evaluate drug release rates.

6. Biological Evaluation:

a) In vitro Studies: Cell viability assays (MTT, Alamar Blue) and cellular uptake studies.

b) In vivo Studies: Pharmacokinetics, bio distribution, and therapeutic efficacy in animal models.

c). Cellular Uptake Studies: Check how well liposomes enter target cells.

d). Hemocompatibility Studies:-

Hemolysis Assay Check RBC damage caused by liposomes

e). Immunogenicity Testing

ELISA Detect antibodies against liposomal components

7. Biocompatibility and Toxicity:

a) Hemolysis Assay: Tests potential for red blood cell damage.

b) Immunogenicity Assessment: Evaluates immune response to liposomes.

8. Drug Release Studies:

a) Dialysis Method: To assess release kinetics under sink conditions.

b) Franz Diffusion Cell: For transdermal drug delivery and membrane permeation.

9. Surface Modification Analysis:

a) XPS & FTIR Spectroscopy: Analyze modifications on the liposome surface.

b) Nuclear Magnetic Resonance (NMR) Spectroscopy:-

^{31}P NMR: Phospholipid head group confirmation.

^{1}H/^{13}C NMR: Structural confirmation of PEG or surface-linked compounds .

c) Dynamic Light Scattering (DLS) with Zeta Potential:- Indirect evidence of surface modification by change in size or charge.

d) Differential Scanning Calorimetry (DSC:-)Detects change in membrane phase transition temperature due to surface-modifying agents.

e) Scanning Electron Microscopy (SEM) / Transmission EM:- Surface morphology visualization.

8. RESULT AND DISCUSSION:-

In this review, an extensive analysis of various research studies on liposomes was conducted. The key findings are summarized as follows:

Structure and Composition: Liposomes are vesicular systems composed of phospholipid bilayers capable of encapsulating both hydrophilic and lipophilic drugs.

Types of Liposomes: Different types of liposomes such as conventional, stealth, cationic, and immunoliposomes were identified and characterized.

Methods of Preparation: Various preparation techniques including thin-film hydration, reverse-phase evaporation, and ethanol injection were compared.

Applications: Liposomes have demonstrated significant applications in cancer therapy, antifungal drug delivery (e.g., Amphotericin B), vaccine delivery (such as mRNA vaccines for COVID-19), and cosmetic formulations.

Advantages and Limitations: While liposomes offer targeted and controlled drug delivery, challenges like short circulation half-life and high production costs were noted.

The review highlights liposomes as a versatile and promising drug delivery system with potential to transform treatment approaches for various diseases. Their biocompatibility and ability to encapsulate a wide range of therapeutic agents make them superior to conventional drug delivery methods.

However, several challenges remain:

Stability Concerns: Liposomes are prone to degradation and drug leakage, which limits their shelf-life and efficacy.

Cost and Scalability: The manufacturing process remains expensive and complex, hindering large-scale production.

Regulatory Hurdles: Obtaining regulatory approval for liposome-based formulations is often lengthy and challenging.

9. SUMMARY CONCLUSION:-

Liposomes have emerged as a revolutionary drug delivery system due to their biocompatibility, ability to encapsulate both hydrophilic and lipophilic drugs, and potential for targeted therapy. Over the years, advancements in liposomal technology have led to improved drug stability, controlled release, and reduced toxicity, making them valuable in pharmaceutical, cosmetic, and diagnostic applications. Despite their advantages, challenges such as high production costs, stability issues, and regulatory hurdles remain. Continued research and technological innovations, including surface modifications and novel formulation techniques, are crucial for overcoming these limitations and expanding their clinical applications. With ongoing advancements, liposomes hold great promise in modern medicine, particularly in cancer therapy, gene delivery, and vaccine formulations, paving the way for more effective and safer treatment strategies.

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