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Review Article

A Comprehensive Review on Liposomes: As A Novel Drug Delivery System

Prachi Moon[1], Pranjali Patil[2], Sabiya Sheikh[3], Soniya Bhongade[4], Priyanka Sakhare[5]

[1] *Assistant Professor, Rasiklal Manikchand Dhariwal Institute of Pharmaceutical Education and Research, Pune.*
[2]*Student, Kishoritai Bhoyar College of pharmacy, New Kamptee, Nagpur.*
[3]*Assistant Professor, Central India College of pharmacy, lonara, Nagpur*
[4]*Assistant Professor, Sushganga College of pharmacy Wani, 445304*
[5]*Assistant Professor, Sushganga College of pharmacy Wani, 445304.*

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ABSTRACT

Liposomes have emerged as one of the most significant and versatile nano carrier systems in modern pharmaceutical sciences due to their remarkable ability to encapsulate both hydrophilic and lipophilic therapeutic agents. These spherical vesicular structures consist of one or more phospholipids bilayers surrounding an aqueous core, enabling them to improve drug solubility, stability, bioavailability, and therapeutic efficacy while minimizing toxicity. Since their discovery by Alec Bangham in the 1960s, liposome's have undergone extensive research and development, leading to several clinically approved formulations for cancer therapy, fungal infections, vaccines, and targeted drug delivery applications. Liposomal systems offer unique advantages such as controlled release, site-specific targeting, prolonged circulation time, and reduced adverse effects. However, challenges including stability issues, high production costs, and scale-up difficulties remain major concerns in their widespread commercialization. This review comprehensively discusses the structure, classification, methods of preparation, characterization, advantages, limitations, applications, marketed formulations, and future prospects of liposome's as novel drug delivery systems. Recent advances in stealth liposomes, ligand-targeted liposomes, stimuli-responsive liposome's, and theranostic applications are also highlighted. Liposome's continued to represent a promising platform for advanced therapeutic interventions and personalized medicine.

INTRODUCTION

Liposome's, derived from the Greek words 'Lipos' meaning fat and 'Soma' meaning body, are

***Corresponding Author:** Prachi Moon
Address: *Assistant Professor, Rasiklal Manikchand Dhariwal Institute of Pharmaceutical Education and Research, Pune*
Email ✉: prachishambharkar100@gmail.com
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spherical concentric vesicles that enclose a water droplet, particularly artificially used to carry drugs into tissue membranes. These round sac phospholipids molecules, which are Nanoparticles (100nm in size), have potential therapeutic properties and are used in various fields such as drug delivery, cosmetics, and biological membrane structure. Liposomes are a tiny bubble with a membrane composed of a phospholipids bilayer, typically made of phospholipids like phosphatidylet-hanolamine and phosphatidylcholine. These phospholipids are amphiphilic with a hydrophilic polar head and a hydrophobic hydrocarbon tail. Their discovery by Bangham in 1961 led to the development of liposome's as a potential carrier for various drugs.

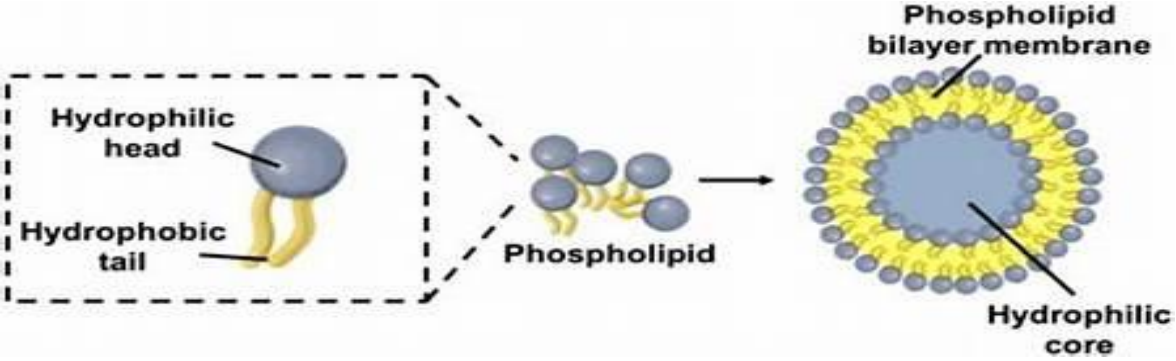

Figure 1: Basic Liposome Structure

Structure of liposomes:

Phospholipids

- Naturally occurring phospholipids used in liposome:
- Phosphatidylethanolamine
- Phosphatidylcholine
- Phsphatidylserine
- Synthetic phospholipids used in the liposomes are:
- Dioleoyl phosphatidylcholine
- Disteroyl phosphatidylcholine
- Dioleoyl phosphatidylethanolamine

Cholesterol

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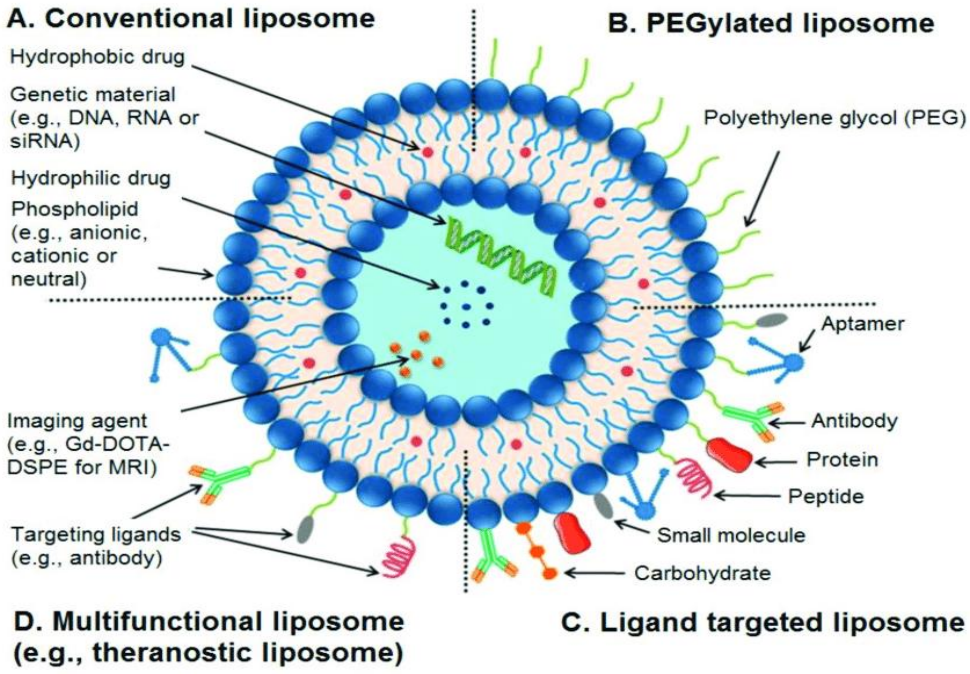

Figure 2: An Illustration of liposome and its structural components

Advantages of Liposome's:

Liposomes significantly enhance vaccine development by enhancing the stability and delivery of antigens, thus enhancing immune responses.

Liposome's enable targeted drug delivery to specific tissues or cells, minimizing exposure to healthy tissues and reducing side effects.

Liposome's can provide sustained therapeutic effects by gradually releasing drugs over time, reducing the need for frequent dosing.

Liposome's enhance the bioavailability and solubility of water-soluble drugs, a crucial factor in drug effectiveness.

- Protection of Sensitive Compounds: Liposome's can protect sensitive drugs or bioactive compounds from degradation due to environmental factors, such as enzymes, pH changes, or oxidation.
- They can be customized in terms of size, composition, and surface modifications to enhance their performance for specific drugs or therapeutic applications.
- Researchers can modify the properties of liposome's, including size, charge, and surface functionality, to suit specific applications.
- Immunogenicity: Liposome's can enhance the immunogenicity of vaccines, resulting in a stronger and more specific immune response.
- Diagnostic Applications: Liposome's are used in diagnostic assays for drug screening, disease detection, and other diagnostic purposes.
- Biocompatibility: Liposome's are generally well-tolerated by the body, making them suitable for various medical and cosmetic applications.

Disadvantages of Liposome's:

Liposome production can be challenging and costly, potentially limiting their widespread use in large-scale pharmaceutical manufacturing.

- Uniformity: Achieving uniformity in liposome size and composition can be difficult, affecting their performance and reproducibility.
- Compatibility Issues: Some drugs may not be suitable for encapsulation in liposomes due to compatibility issues, limiting the range of drugs that can benefit from liposomal delivery.
- Regulatory Approval: Obtaining regulatory approval for liposomal drug products can be a complex and time consuming process, adding to the development timeline and cost
- Niche Applications: Liposome's may not be suitable for all drug delivery needs, and alternative delivery systems may be preferred in certain cases.
- Short Circulation Half-Life: Liposome's can be rapidly cleared from the bloodstream by the body's immune system, limiting their time window for drug delivery.
- Storage Stability: Liposome's can be prone to instability during storage, leading to aggregation, leakage of encapsulated substances, or changes in size and structure
- Expense: Producing liposomal formulations can be costly, which may lead to higher drug prices for liposome based therapies.
- The intricate nature of liposomal formulation development necessitates specialized expertise, potentially limiting the accessibility of these products to researchers and manufacturers.

Classification of Liposome's:

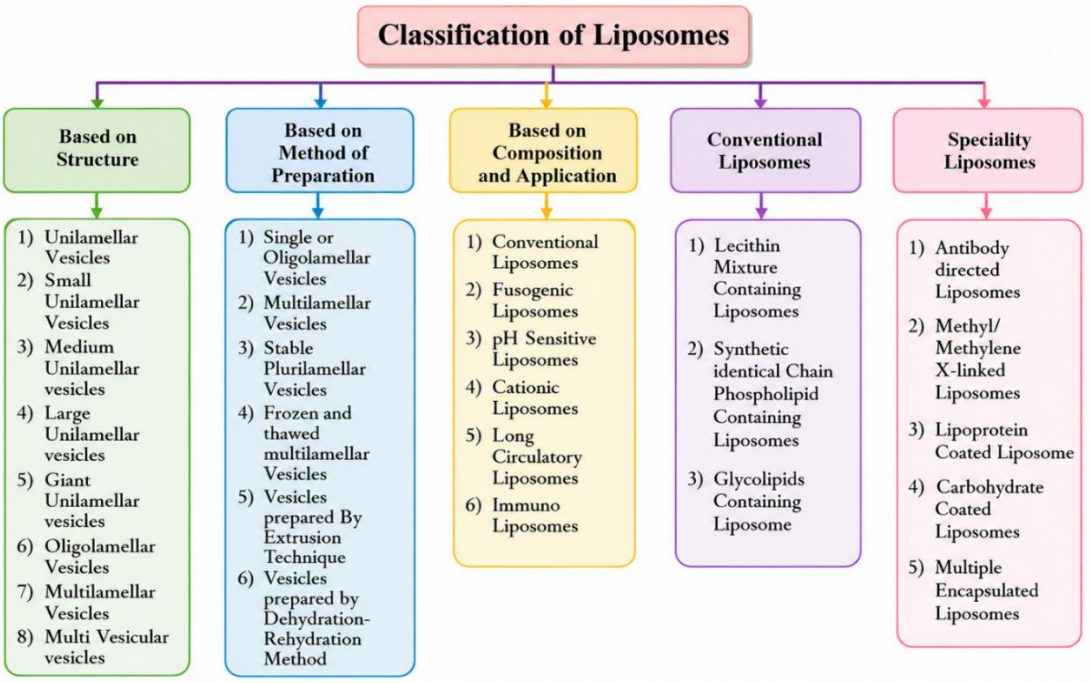

Figure 3.Classification of Liposome's

Mechanism of formation of Liposome's:

Liposome performs their motion by four distinct Mechanism-

- Endocytosis – This take location via phagocytes' cells of reticuloendothelial system together with neutrophils.
- Adsorption – It occurs to the cellular surface through non precise electrostatic forces or by using interplay with cell surface additives.

- Fusion- It takes place by means of the insertion of liposomal bilayer into plasma membrane with continuous release of liposomal content into the cytoplasm.
- Lipid exchange- on this transfer of liposomal lipids to the cellular membrane without association of liposomal contents.

Method of preparation

The methods can be classified broadly into two categories: mechanical dispersion method sand solvent dispersion methods.

1. Mechanical Dispersion Methods

a) Thin-Film Hydration (Bangham Method)

This is the most common and simple method of preparing liposome's.

Process:

1. Dissolve phospholipids in a volatile organic solvent (e.g., chloroform or methanol).
2. Remove the solvent by rotary evaporation to form a thin lipid film on the walls of a round-bottom flask.
3. Hydrate the lipid film by adding an aqueous buffer (e.g., PBS) with gentle agitation, which leads to the formation of multilamellar vesicles (MLVs).
4. Subject the MLVs to further processing (e.g.,
5. sonication, extrusion) to obtain smaller unilamellar vesicles (SUVs) or large unilamellar vesicles (LUVs).

Applications

Widely used for basic research, encapsulation of both hydrophilic and hydrophobic drugs.

b) Sonication

This method reduces the size of liposomes (MLVs) prepared by the thin-film hydration method.

Process:

1. The MLV suspension is sonicated using either a probe-type or bath-type sonicator.
2. The mechanical energy breaks the larger vesicles into smaller SUVs (20-100 nm).

Applications:

Suitable for forming small liposomes, but sonication can lead to degradation of phospholipids and encapsulated drugs.

c) Extrusion

Extrusion is used to achieve uniform liposome size by forcing them through polycarbonate membranes with specific pore sizes.

-Process:

1. Pass the MLV suspension through membranes under pressure.
2. This produces LUVs with a more uniform size distribution.

Applications:

Preferred for producing large, uniform liposomes for drug delivery.

2. Solvent Dispersion Methods

a) Ethanol Injection Method

In this method, lipids dissolved in ethanol are injected rapidly into an aqueous solution.

Process:

1. Dissolve lipids in ethanol.

2. Inject the ethanol solution into an aqueous phase under rapid stirring.

3. The lipid molecules self-assemble into liposomes due to the sudden change in solvent polarity.

Applications:

Simple and quick, but the presence of residual ethanol can be a limitation.

b) Reverse-Phase Evaporation Method (REV)

This method is useful for encapsulating a large volume of aqueous solution into liposome's.

Process:

1. Dissolve phospholipids in an organic solvent (e.g., ether).
2. Add an aqueous phase and form a water-in-oil emulsion by sonication.
3. Evaporate the organic solvent under reduced pressure, which causes the emulsion to collapse into liposomes.

Applications:

Useful for encapsulating large aqueous volumes and proteins.

c) Solvent-Spherule Method

This method involves dissolving lipids in an organic solvent, which is then emulsified into an aqueous solution.

Process:

1. Lipids are dissolved in an organic solvent such as chloroform.
2. The lipid solution is then emulsified into an aqueous solution.
3. Upon removal of the organic solvent, liposome's are formed.

3. Detergent Removal Methods

This method involves the formation of liposomes by the removal of detergents that solubilise lipids.

a) Dialysis

Process:

1. Phospholipids are first dissolved in a detergent solution (e.g., Triton X-100).

2. The detergent-lipid micelle solution is placed in a dialysis bag and dialyzed against a detergent-free buffer.

3. As the detergent is gradually removed, liposomes form spontaneously.

Applications:

Suitable for producing high-quality liposome's without shear stress.

Marketed formulations of Liposome's:

Table 1 : Commercially Available Liposomal Drug Formulations and Their Therapeutic Applications

Brand Name	Drug	Indication	Type of Liposome
Doxil/Caelyx	Doxorubicin	Breast cancer, Ovarian cancer, AIDS-related Kaposi's sarcoma	PEGylated Liposome
Doxil/Caelyx	Amphotericin B	Fungal infections, Leishmaniasis	Liposomal Amphotericin B
DepoDur	Morphine sulfate	Post-operative pain management	DepoFoam technology (multi-vesicular)
Marqibo	Vincristine sulfate	Acute lymphoblastic leukemia (ALL)	Sphingomyelin/cholesterol-based liposome
Onivyde	IRinotecan	Metastatic pancreatic cancer	Liposomal formulation

Vyxeos	Daunorubicin and Cytarabine	Acute myeloid leukemia (AML)	Dual-drug liposome formulation
Visudyne	Verteporfin	Age-related macular degeneration (AMD), Pathologic myopia	Liposomal formulation
Myocet	Doxorubicin	Metastatic breast cancer	Non-PEGylated liposomal doxorubicin
DepoCyt	Cytarabine	Lymphomatous meningitis	Sustained-release liposomal formulation
MEPACT	Mifamurtide	Non-metastatic osteosarcoma	Liposomal formulation
Inflexal V	Influenza vaccine	influenza prophylaxis	Liposomal adjuvant vaccine

Evaluation of Liposome's:

1. Particle Size and Size Distribution

Particle size is crucial as it influences the circulation time, tissue distribution, and cellular uptake of liposomes.

➢ **Techniques:**

- **Dynamic Light Scattering (DLS):** Measures the hydrodynamic diameter of liposome's in solution and provides a size distribution profile.
- **Electron Microscopy (TEM/SEM):** Provides direct visualization of liposome size and shape.
- **Nanoparticles Tracking Analysis (NTA):** Measures the size and number of individual particles based on Brownian motion.
- **Importance:** Small liposome's (<100 nm) generally exhibit prolonged circulation, while larger liposome's tend to accumulate in the liver and spleen (RES clearance).

2. Zeta Potential (Surface Charge)

Zeta potential measures the surface charge of liposome's and is critical for predicting the stability of the formulation.

➢ **Techniques:**

- **Zeta Potential Analyzer:** Uses electrophoresis light scattering to measure the charge on the surface of liposome's.
- **Importance:** A highly positive or negative zeta potential (≥ ±30 mV) indicates good electrostatic stability and prevents aggregation due to repulsion forces. Neutral or slightly charged liposome's may aggregate over time.

3. Encapsulation Efficiency (EE %)

Encapsulation efficiency evaluates the percentage of drug that is successfully encapsulated within the liposome.

➢ **Techniques:**

- **Ultracentrifugation/Dialysis:** Separates free drug from liposome-encapsulated drug.
- **HPLC or UV Spectroscopy:** Used to quantify the amount of encapsulated drug after separation.
- **Importance:** High encapsulation efficiency is desirable for drug delivery to minimize wastage and enhance therapeutic efficacy.

4. Drug Release Profile

Evaluating the release profile of the drug from liposome's is crucial to understand the kinetics and ensure controlled delivery.

➢ **Techniques:**

- **In vitro Release Studies:** Liposome's are incubated in conditions mimicking physiological environments (pH, temperature) and sampled over time to measure drug release.
- **HPLC or UV Spectroscopy:** Quantifies drug release at specific time points.
- **Importance:** The release profile must be controlled to prevent burst release and ensure sustained delivery at the target site.

5. Morphology and Lamellarity

The morphology and number of bilayers (lamellarity) affect encapsulation, release, and the interaction of liposomes with biological membranes.

➢ **Techniques:**

- **Cryo-TEM:** Visualizes liposome structure in near-native conditions, including lamellarity.
- **Freeze-Fracture Electron Microscopy:** Provides detailed images of liposome bilayers.
- **Importance:** Multilamellar vesicles (MLVs) offer different drug release kinetics compared to unilamellar vesicles (SUVs), influencing therapeutic action.

6. Stability

Stability studies assess the physical and chemical integrity of liposomes over time under various conditions.

➢ **Techniques:**

- **Size and Zeta Potential Monitoring:** Monitors changes in liposome size or charge to detect aggregation or instability.
- **Oxidation and Hydrolysis Testing:** Measures degradation of lipids, especially oxidation of unsaturated fatty acids.
- **Temperature Stability Studies:** Liposomes are subjected to different temperatures to assess shelf-life.
- **Importance:** Stability is critical for ensuring that liposomes retain their therapeutic properties during storage and transport.

7. Pharmacokinetics and Bio distribution

Evaluating the pharmacokinetics and bio distribution of liposome's is crucial to understand their in vivo behaviour, including circulation time, tissue targeting, and clearance mechanisms.

➢ **Techniques:**

- **Fluorescence or Radioactive Labelling**: Liposome's are labelled with fluorescent or radioactive markers to track their distribution in animal models.
- **Blood Sampling and Tissue Analysis**: Quantifies the amount of liposome's in the bloodstream and various organs over time.
- **Importance:** PEGylated liposome's, for example, have prolonged circulation times due to reduced recognition by the reticulo endothelial system (RES).

Application for Liposome's

Drug Delivery:

- Liposome's are commonly used as drug delivery vehicles to encapsulate and deliver both hydrophobic and hydrophilic drugs.
- They can improve drug solubility, stability, and bioavailability.
- Liposomal drug formulations can target specific tissues or cells, reducing systemic side effects.
- ***Vaccines:***
  Liposome's are used as adjuvants or carriers for vaccines to enhance immunogenicity.
  They can improve antigen delivery to immune cells, leading to a stronger immune response.

- ***Cosmetics and Skincare:***
  Liposomes are utilized in cosmetics and skincare products for controlled release of active ingredients, such as vitamins and antioxidants.
  They can enhance the penetration of ingredients into the skin, improving their efficacy.
- ***Gene Delivery***
  Liposomes can be used to deliver genetic material, including DNA and RNA, for gene therapy applications.
  They protect and facilitate the transport of genetic cargo into target cells.
- ***Diagnostics:***
  Liposomes can serve as carriers for contrast agents in medical imaging, such as magnetic resonance imaging (MRI) and ultrasound.
  They enable targeted imaging of specific tissues or cells.
- ***Cancer Therapy***
  Liposomal formulations of chemotherapy drugs, like Doxil (liposomal doxorubicin), are used to treat cancer.
  They can improve drug circulation time and reduce damage to healthy tissues.
- **Food Technology**
  Liposomes are applied in the food industry for encapsulating and protecting sensitive ingredients, such as vitamins, flavors, and antioxidants.
  They can improve the stability and bioavailability of these additives in food products.

Biotechnology

- Liposomes are used in research and biotechnology applications for drug screening and delivery to cells in vitro.
- They are valuable tools for studying cell membrane interactions and drug transport mechanisms.

Transdermal Drug Delivery:

- Liposomal formulations can be applied topically to deliver drugs through the skin.
- They offer controlled release and can avoid the first-pass metabolism in the liver.

Personal Care Products

- Liposomes are employed in personal care products such as sunscreens and moisturizers to enhance the delivery of active ingredients.

Veterinary Medicine

- Liposomes are used in veterinary medicine for drug delivery to animals, similar to their applications in human medicine.

Environmental Remediation:

- Liposomes can be utilized for the controlled release of remediation agents in environmental cleanup efforts.

Intracellular Delivery:

- Liposomes are valuable tools in research for delivering molecules into specific organelles within cells.

Nutraceuticals

- Liposomes are used to enhance the bioavailability of nutraceutical compounds in dietary supplements.

Wound Healing

- Liposomal formulations can be applied to wound dressings to promote the controlled release of wound-healing agents.

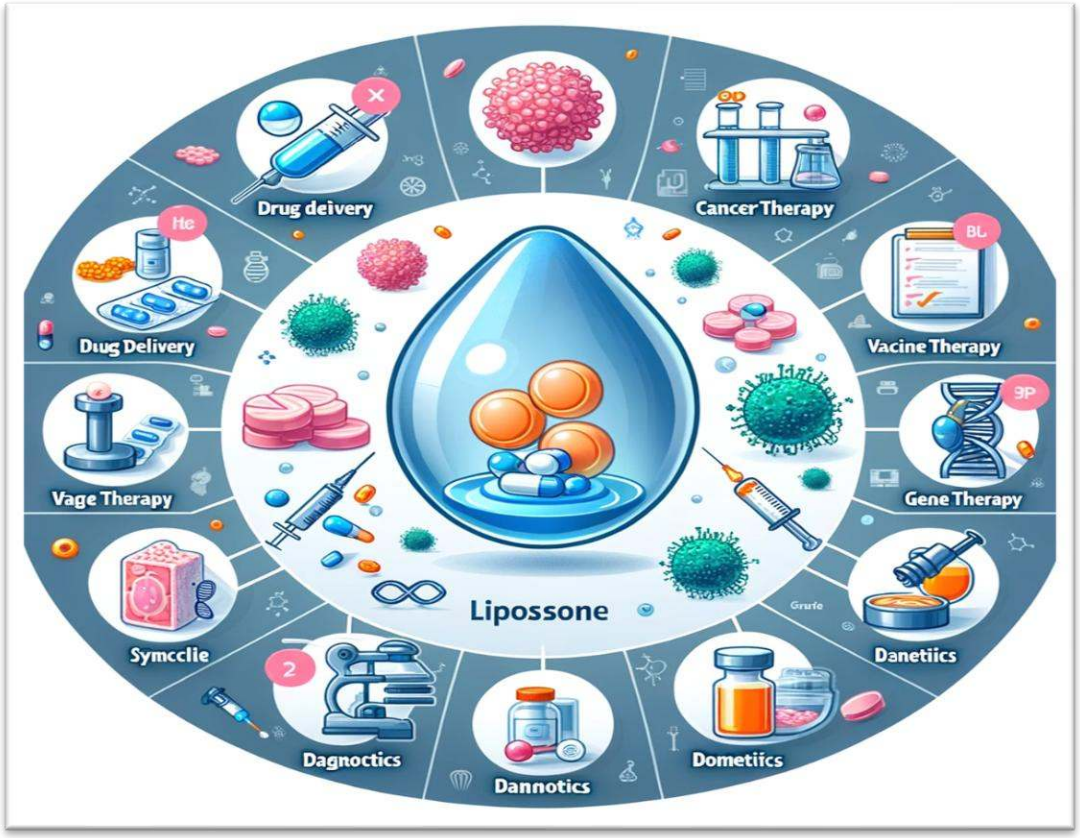

Figure 4: Application for Liposomes

Recent Approaches in Liposome Research

Below are some of the prominent approaches:

1. Targeted Liposomal Delivery Systems

- **Ligand-conjugated liposomes**: Recent studies have focused on attaching ligands (e.g., antibodies, peptides, or small molecules) to the surface of liposomes to target specific cells or tissues. This approach enhances drug accumulation at the desired site, minimizing off-target effects. For example, HER2-targeting liposomes for breast cancer treatment are being developed using trastuzumab as a ligand.
- **pH-sensitive liposomes:** These are designed to release their payload in response to the acidic environment of tumors or intracellular compartments (such as endosomes and lysosomes). Such liposomes remain stable in the bloodstream but release their contents once they encounter a lower pH.

2. Stimuli-Responsive Liposomes

- **Thermo-sensitive liposomes**: Liposomes that release their payload in response to increased temperature have gained traction. These formulations can be used in combination with hyperthermia (heat therapy) to trigger localized drug release at tumor sites
- **Magnetic liposomes**: Incorporating magnetic nanoparticles into liposomes allows for drug delivery under the guidance of an external magnetic field. This approach helps concentrate the therapeutic agent at the disease site while minimizing systemic exposure.
- **Ultrasound-responsive liposomes**: These formulations allow for controlled drug release using ultrasound waves, which can non-

invasively trigger liposomal drug release in specific tissues.

4. Immunoliposomes

Immunoliposomes, which are antibody-conjugated liposomes, are designed for targeted drug delivery to cancer cells or other disease-specific sites. By attaching monoclonal antibodies to the surface, these liposomes can specifically bind to antigens overexpressed in certain diseases, particularly cancers.

Example: Anti-CD19 immunoliposomes for targeted therapy of B-cell malignancies.

4. Liposomal Vaccines

Liposomes are now being explored as carriers for vaccines. They offer protection of the antigen, ensure slow release, and enhance immune responses. Some COVID-19 vaccine candidates have utilized liposomal technology to deliver mRNA effectively.

Example: The development of liposomal mRNA vaccines for infectious diseases such as COVID-19, which leverage lipid nanoparticles for encapsulating mRNA, as seen with the Pfizer-BioNTech and Moderna vaccines.

5. Liposomal Gene Therapy

Liposomes are being utilized to deliver gene-editing tools like CRISPR-Cas9 to target specific genes in diseases. Cationic liposomes, which carry a positive charge, are especially effective in encapsulating and delivering negatively charged nucleic acids (like DNA or RNA) to cells.

CONCLUSION

Liposomes are an innovative drug delivery system with potential applications in pharmaceuticals. Research has shown their ability to overcome challenges from traditional methods, enhancing therapeutic efficacy and safety. Despite challenges, continued innovation in liposomal technologies holds great promise for the future of drug delivery in the pharmaceutical industry. Liposomes offer a versatile approach to drug delivery, improving efficacy, reducing side effects, and enabling precise therapy targeting. Further advancements in liposomal technology are expected to expand their use in various medical applications.

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