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AN UPDATED REVIEW ON LIPOSOMES

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Abstract

Liposomes, tiny bubbles made from the same material as a cell membrane, are considered the most successful nanocarriers for drug delivery. They are biocompatible and stable, encapsulating both hydrophilic and lipophilic drugs and protecting them from degradation. Liposomes are the most commonly used drug delivery vehicles due to their high drug trapping capacity and efficiency. These offer controlled release, targeted drug delivery, increased therapeutic efficacy, and reduced dosing frequency. Liposome-based drug formulations have been approved for clinical use and are the subject of extensive research. Recent liposomal formulations aim to reduce toxicity and increase target site accumulation. With numerous advantages, liposomes prove their uniqueness in safe drug delivery in various areas of pharmacy and medicine. We will keep an eye on overall updated review on liposomes classification, their structural components, mechanism of action, stability, method of preparation, their evaluation tests and applications.

Keywords: Liposomes, Vesicles, Drug Delivery, Cholesterol, Phospholipids, dds.

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Introduction

British hematologist Alec Douglas Bangham discovered the liposome in 1961 at the Babraham Institute in Cambridge. He and his associate Gerald Weissman proposed simpler phospholipid bilayer vesicles, known as liposomes, for use in medicine delivery. Liposomes adsorb both lipophilic and hydrophilic substances, preventing degradation and releasing active components gradually. The term "liposome" comes from the Greek words "Lipos" meaning fat and "Soma" meaning body. Liposomes, microscopic bubbles or vesicles, are composed of the same substance as a cell membrane and can grow in various sizes [1, 2]. They were first characterized by British Hematologist Dr. Alec D. Bangham in 1964 at the Babraham Institute. They offer prolonged drug release and lower systemic toxicity compared to free medications. Liposomal amphotericin B injection is highly effective against black mycosis, an uncommon disease affecting the lungs, stomach,

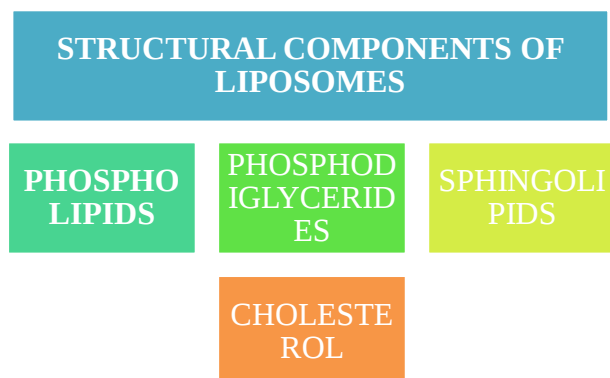
intestines, skin, sinuses, and brain. The qualities of the carrier (liposomes) also influence drug distribution, absorption, metabolism, and excretion.

Liposomes are self-closing spherical structures made of lipid bilayers that partially encapsulate the surrounding fluid. They are widely used in the pharmaceutical and cosmetics sectors due to their unique properties [3-8]. The liposomes are the bilayer structures. Micelles are the name for the monolayer structures [9]. The development of liposome nanoplateforms has led to significant advancements in manufacturing techniques [10].

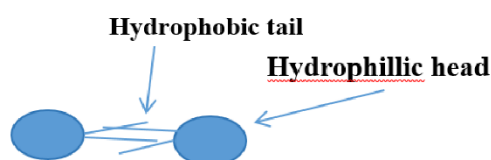
Raw Materials in Liposome Preparation

Amphiphilic substances containing both water-hating and water-friendly portions are called lipids. Liposomes are made up of one or more lipid bilayers that interact with the aqueous phase in a hydrophilic and hydrophobic manner. Water molecules reject the hydrophobic portions of liposomes, or tails, which causes the liposomes to self-assemble [6]. Moreover, polymers may be utilized to create polymericosomes, which are novel medication and gene carriers [11].

The following make up liposome structure



Phospholipids



Phospholipids that include glycerol are most often utilized in liposome compositions. phospholipids The primary structural element of biological coverings is made up of phospholipids, which are classified as either **PHOSPHODIGLYCERIDES** And **SPHINGOLIPIDS** [9]

This alteration results in more empty spaces between the phospholipids, which helps with the liposome preparation process' film production stage[12].

The following types of phospholipids are utilized in liposomal preparation:

- Natural phospholipids
- Synthetic phospholipids
- Semisynthetic phospholipids
- Modified natural phospholipids

Examples

Phosphatidyl choline, Phosphatidyl inositol Phosphatidyl ethanolamine, Phosphatidyl glycerol.[13]

➤ Cholesterol

The addition of cholesterol enhances the characteristics of lipid bilayers. Phosphatidylcholine to cholesterol ratios can range from 1:1 to 1:2. [14]. Although hydrophobic and distinct head group interactions have been linked to the high solubility of cholesterol in phospholipid liposomes, the organization of cholesterol in the bilayer is unclear [9]. Liposomes often have a less rigid or fluidic bilayer when cholesterol is absent, which causes these drug carriers to become unstable. Hence, cholesterol is utilized in the lipid component of liposome synthesis and is a part of it [12].

Classification of Liposomes

By shielding the loaded medications from the host organism's harmful chemicals, the hydrophilic inner portion of the liposome helps reduce unfavorable side effects [15]. Liposomes can be classified architecturally into many categories:

- **Based on the method of preparation [9]**
 - a) Reverse phase evaporation vesicles(REV)
 - b) Multi lamellar vesicle by REV(MLV-REV)
 - c) Dehydration- rehydration method(DRV)
 - d) Vesicle prepared by extraction method(VET)
 - e) Stable plurilamellar vesicles(SPL)
 - f) Frozen and thawed MLV(FAT-MLV)
- **Depending upon size**

1. Multilamellar Liposomes

Liposomes known as Multilamellar Vesicles (MLV) are made up of many concentric lipid bilayers that vary in size from 0.1 to 0.5 μm . MLVs are structured like an onion.

2. Unilamellar Liposomes

A single bilayered phospholipid sphere encircling the aqueous solution is present in vesicles. Small unilamellar vesicles (SUVs), with sizes ranging from 0.02 to 0.05 μm , are a subset of unilamellar vesicles.

3. Multivesicular Liposome

A multivesicular vesicle (MVV) is a vesicle made up of many non-concentric vesicles encircled by a single bilayer. Multifunctional liposomes ranging in size from 2 μm to 40 μm may be the source.

4. Oligolamellar Liposome

Compared to multilamellar liposomes, oligolamellar liposomes have fewer layers of plaque. They range in size from 0.1 to 10 μm [12, 20].

5. Giant unilamellar Liposomes(GL)

These liposomes, which range in size from 10 to 1000 μm , are the biggest. They may be LUVs or SUVs [17].

Sterically stabilized liposomes were created to boost the liposomes' stability and lengthen their duration circulating in the blood [18].

Characteristics of liposomal drug delivery system

1. The pharmaceutical industry is beginning to recognize liposomes as one of the potential medication delivery systems.
2. Specified physical and chemical features of liposomes as a drug delivery vehicle have a significant impact on the properties of nanomedicines, including drug circulation and absorption into bodily membranes, as well as release from dosage forms at specified places.
3. The word "zeta potential" refers to the electrokinetic potential in colloidal systems, which significantly influences the different characteristics of liposomes.[19,20]

Mechanism of Action of Liposomes [20, 21]

A zone of aqueous solution surrounded by a hydrophobic membrane makes up a liposome. Hydrophobic compounds readily dissolve in lipid

membranes, allowing hydrophilic and hydrophobic molecules to be transported via liposomes.

Advantages

1. They are adaptable enough to pair with ligands unique to certain sites to accomplish active targeting.
2. Long-term medication release is possible with liposomes.
3. They shield the medications within from deteriorating in unfavorable conditions.
4. The pharmacokinetic and pharmacodynamic properties of encapsulated medications can be altered by liposomes [12].
5. Liposome is non-toxic, fully biodegradable, immunogenic, and biocompatible [22].

Disadvantages

1. Less stable liposomes exist.
2. Following an IV injection, they are quickly eliminated from the circulation by reticuloendothelial system cells.
3. Phagocytes have an impact on the delayed release of drugs.
4. Limited solubility [23].
5. The carrier lipids are subject to oxidation and hydrolysis.[12]
6. Less steady [23]
7. Drug/molecule encapsulation leakage and fusing [12].
8. High production costs [23].

Stability of Liposomes

- The stability of the proposed formulation is crucial in the creation of liposomal medicines. The stability of the liposomes throughout production, storage, and distribution is critical to the drug's therapeutic efficacy. The drug's chemical and physical integrity must thus be tested as part of a stability process before it is stored [10, 20].

Physical Stability

Several methods, including light scattering and electron microscopy, can be employed to track this by evaluating the bubbles' size and visual characteristics [24].

Chemical stability

Phospholipids' chemical stability is crucial since they usually serve as the bilayer's structural support. The performance of phospholipid bilayers can be impacted by two different types of chemical breakdown reactions: phospholipid peroxidation and phospholipid hydrolysis. [25].

a) Phospholipid peroxidation: By employing premium starting materials that have been separated from hydro peroxides and transition metal ions, phospholipid peroxidation in liposomes can be reduced. Oxidation is prevented by low storage temperatures and shielding from light and oxygen. To further enhance defense against oxidation, antioxidants such butylated hydroxytoluene (BHT) and alpha-tocopherol can be added [24, 20].

Methods of Preparation

➤ Conventional Preparation Methods
a) Mechanical dispersion method

1. Hand Shaking Method
2. Sonication Method
a) Probe sonication method
b) Sonication Bath
3. Micro emulsification
4. French Press Method
5. Freeze Thawed Liposomes
6. Membrane Extrusion
b) dispersion method
1. Solvent injection method
2. Thin Film Hydration Method
3. Double Emulsion Method
4. Reverse Phase Evaporation Methods
➤ Novel Preparation Methods
1. Liposome Preparation Using Supercritical Fluid Technology
2. Reversed Phase Separation Methods

➤ Conventional Preparation Methods

The following is a description of the liposome preparation methods:

A) Mechanical dispersion method

This approach entraps 5–10% of the water, which is a minor portion of the overall volume required for swelling. As a result, during swelling, a significant portion of the water-soluble molecule is lost; in contrast, the oil-soluble chemical can encapsulate up to 100% [20].

1. Hand Shaking Method:

This method involves dissolving the lipid in an organic solvent (mostly ethanol) in a flask with a circular bottom while stirring continuously [10].

a) **Sonication Method:** This is the most widely used process, which involves using a rotary evaporator and manual stirring to create SUV from MLV.[20]

b) **Sonication Bath:** This approach involves placing a liposome dispersion in a container under an inert environment or into a sterile vessel with a temperature control system [10].

2. **Micro emulsification:** Using high shear produced by a high-pressure homogenizer, a lipid composition is micro-emulsified to make tiny vesicles in this process. These techniques are applied in the commercial production of tiny lipid vesicles.[20]

3. **French Press Method:** This method is straightforward, efficient, and repeatable; it also involves handling delicate materials with caution. The MLV is expelled during this procedure at 4°C and 20,000 pressure through a tiny opening. Furthermore, it features many focus areas in terms of sound reinforcement technology.[27]

a. Solvent dispersion method:

1. **Solvent injection method:** Small unilamellar liposomes spontaneously develop when lipid materials and lipophilic compounds are dissolved in a water-miscible organic solvent and the organic phase is then injected into a large volume of an aqueous buffer [20].

2. Thin Film Hydration Method: For loading lipophilic medicines, the conventional approach of thin layer hydration is favored. Aqueous solutions can be added to MLV suspensions to hydrate the lipid layer [28].

3. Double Emulsion Method: Two-step emulsion preparation was used to create W1/O/W2 double emulsions at room temperature (25°C). Many water-impermeable organic solvents (or mixed solvents) can be utilized as the oil phase (O), including ether and chloroform; however, cyclohexane was used selectively because of its melting point being near to the waterfall melting temperature [29].

➤ Novel Preparation Methods

The main goal of new liposome manufacturing techniques is to enable larger industrial production and application of liposomes to a variety of phospholipids and medications [30].

1. Liposome Preparation Using Supercritical Fluid Technology

Non-condensable liquids known as supercritical fluids have the ability to weigh more than necessary and become exceptionally dense at positive temperatures. Compared to ordinary liquids, supercritical fluids have numerous unique properties because the process that separates the liquid and fuel phases vanishes [20].

2. Reversed Phase Separation Methods

Methods of Reversed Phase Separation Since this method was thought to be the first to produce liposomes with a high liquid-lipid area ratio and the capacity to extract a sizable portion of the promised aqueous material, it allowed liposomal innovation to progress. The quantity of materials categorized as natural solvents and the brief sonication periods present the biggest challenges to this method [31, 20].

Evaluation of Liposome

1. Particle Size Analysis

On a glass slide, a drop of the liposomal formulation was evenly distributed, and it was left to dry overnight. Following the application of platinum coating on the samples using a Polar sputter coater on E5100 (Polaron, England), the samples were examined at an accelerating voltage of 20 kV using a Philips 505 scanning electron microscope. When necessary, photos were taken with magnifications of 70x, 100x, 200x, and 300x [20].

2. Percentage of Entrapment efficiency [20]

It is calculated using the following formula, which expresses the ratio of the entrapped drug (mg) to the total drug

$$\text{Entrapment efficiency} = \frac{\text{amount of drug entrapped}}{\text{total amount of drug}} \times 100$$

3. In-vitro drug release study from liposomes:

A test tube with a 20 mm diameter hole held 5 ml. A semipermeable dialysis membrane was placed over the open end and secured with string. With the membrane touching the water's surface, the tube was inverted and placed over the top of 100 mL of water in a 250 mL beaker. A clamp fastened to a stand held the tube in

place. To keep a vortex from forming in the beaker, the water was agitated using a magnetic stirrer. At 37°C, the temperature was kept constant. The medication that is liberated from the liposomes penetrates the membrane and reaches the receptor chamber's center. A UV spectrophotometer was used to measure the absorbance at 263 nm using aliquots of 2 ml from the receiving chamber's medium, which were then suitably diluted and compared to a new medium blank. To maintain a constant medium volume in the beaker, 2 ml of fresh medium was added at the same time [32,20].

4. Vesicles Shape:

Electron microscopy was utilized to analyze the morphology of vesicles [20].

5. Trapped Volume:

This is a crucial liposome-related parameter. It is equal to the volume of lipids in the water times the volume trapped in it. Between 0.5 and 30 microliters/micromole are possible ranges [20].

Applications of liposomes

Applications of liposomes in medicine and pharmacology can be categorized into two main categories: studies on cellular interactions, recognition processes, and the functioning of certain substances; and diagnostic and therapeutic applications of liposomes containing different markers or drugs and their use as an instrument, model, or reagent [33].

1. Using liposomes to treat HIV infections Numerous analogues of antiretroviral nucleotides have been created to treat individuals with acquired immune deficiency syndromes (AIDS). One of these is antisense oligonucleotide, a novel antiviral medication with potential for use in treating HIV-1 [9].
2. Topical drug delivery: It has been demonstrated that liposome application on the skin's surface is successful. Liposomes enhance the skin's permeability to a range of entrapped drugs while also reducing the need for higher dosages of these drugs, which lessens their side effects [9].
3. Enhanced antimicrobial safety and efficacy: There are two reasons why antimicrobial agents have been placed inside liposomes. They first guard against enzymatic degradation of the drug that is entrapped. Penicillins and cephalosporins, for example, are susceptible to the degradative activity of β -lactamase, which is generated by specific microbes [9].
4. Longer release, less toxicity, less local irritation, and improved stability in a big aqueous core [9].
5. Liposome-containing droplets are produced in an aerosol using inhalation devices such as nebulizers [9].

6. Liposomes in Treatment with Nucleic Acid

Delivering nucleic acid to cells both in vitro and in vivo is essential for recombinant DNA technologies, gene function research, and gene therapy. It has been reported that pH-

sensitive liposomes can be used as plasmid expression vectors to deliver DNA into the cytosol [20].

7. Liposomes in Ocular Disorders

In developed nations, retinal disorders are the main cause of blindness. The liposome serves as a vehicle for both monoclonal antibodies and genetic transfection. Currently licensed liposomal medications are "carriers" for use in the eye; the advantages of the liposome will be applied in ophthalmic therapy, diagnosis, and research in the future [34].

8. Liposomes in the Therapy of Cancer

This prevents off-target toxicity brought on by the EPR effect. The FDA has approved certain liposome-based DDSs that exhibit strong antitumor activity [35].

9. The Use of Liposomes in Ophthalmic Therapy

Liposome transporters are an innovative method of delivering drugs to the eye. The maximal EE of the nano-scale medication is obtained, resulting in no eye discomfort. The new formulations also improve patient compliance, making the promising for ocular drug delivery [36].

10. Liposomes in infections and parasitic illnesses

Liposomes in Illness and Infections with Parasites Macrophages can be trained to target specific sized liposomes as living targets [37].

Table 1: The various liposomal formulation for commercial use [1]

Drug	Product	Indications
Doxil™	Doxorubicin	Refractory Kaposi's sarcoma, recurrent breast cancer, and ovarian cancer
Myocet®	Doxorubicin	Recurrent breast

Conclusion

Liposomes are a promising carrier for improving site-targeted drug delivery, reducing repeated administration, increasing therapeutic value, and reducing toxicity. They are used in various pharmaceutical and pharmacology applications, including therapeutic and diagnostic purposes, and gene delivery. Liposomes must be sterile, pyrogen-free, and can fuse spontaneously with cells for effective delivery. They have been used in pharmaceutical and cosmetics industries, targeting potent drug candidates with low therapeutic indications to the diseased site. Liposomes were the first nanotechnology-based drug delivery systems approved for clinical applications due to their biocompatibility and biodegradability. They can be prepared from synthetic and naturally occurring phospholipids, and their preparation methods can influence particle structure and drug entrapment. Liposomes are tools for drug targeting in biomedical situations and can reduce dose-related drug toxicity.

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Conflicts of Interests

There are no conflicts of interest.

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Authors Contributions

All the authors have contributed equally.

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