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Review Article

Liposomes as Drug Delivery System: An Updated Review

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Abstract

The liposomes were the first Nano medicine to be accepted for clinical use. They are the spherical vesicles that possess mid empty aqueous space, which is encircled by a phospholipids bilayer. Liposomes have immense capability to prevent the degradation of drugs, reduce side effects and are thus increasingly used for targeted drug delivery. The drugs can either be incorporated inside the aqueous space (hydrophilic drugs) or inside the phospholipids bilayer (hydrophobic drugs) of liposomes for the targeted drug delivery. Considering the importance of liposomes as a drug delivery system, the present review paper tries to look into its details. The entire paper is classified into six parts. The first part is introductory. The second part discusses the classification of liposomes. In the third segment, the structural components of liposomes are detailed. The fourth portion of the paper talks about methods of preparation of liposomes. In the fifth segment, the characterization of liposomes is discussed. The sixth part discusses the application of liposomes and the last part is given to concluding observation. Literature shows distinct types of liposomes, categorized based on size, number of lipid layers, composition and preparation method. They are recently used for various nanoscale drugs formulation and a piece of concrete evidence was seen recently in recommended drug for black fungus i.e., Liposomal Amphotericin B. Although, their development and application are remaining the challenge due to costly and tedious processes involved in their production and development. Therefore, further research and development are required to perform to overcomes these challenges.

Keywords: Liposome, characterization, amphiphatic, controlled release, phospholipids

INTRODUCTION

In comparison to conventional drugs, the primary purpose of a targeted drug delivery is to efficiently distribute a drug directly to the site of action to achieve better efficacy and to reduce adverse effects. Amongst numerous carrier system, liposomes have generated an excellent interest as a result of their versatility. Liposomes are a desirable delivery method because of their adaptable physicochemical and biophysical characteristics, which allow for simple modification to meet a variety of delivery needs. Liposomes are bilayered vesicles that are concentric in shape, having a diameter of 0.01–5.0 µm as it may be made from cholesterols, glycolipids, non-harmful surfactants, long chains of fatty acids, sphingolipids and even membrane protein.[1] Liposomes are vesicular structures that develop when phospholipids are spread in water and have an inner aqueous phase enclosed by phospholipid bilayer membranes. The liquid interior was encased in a sphere-like container, which contained substances like peptides & protein, antibiotic, enzymes, hormones, antimycotic & antitumor agents and even plasmids.[2] Liposomes mostly consist of biocompatible and biodegradable materials capable of encapsulating both hydrophobic and hydrophilic molecules on one platform. The ongoing researches focus more on the growth of long circulating stealth liposomes and multi-functional liposomes with advanced in vitro properties.[3]

Liposomes were discovered 54 years ago by British hematologist Dr. Alec D Bangham that possesses the multi-faceted tool in medicine, biology and biochemistry nowadays. In the year 1960 liposome were used as a transporter in its aqueous compartment to transport a wide range of compounds. It is possible to develop and process liposomes varying in size, structure, charge and lamellarity. At present anti-tumor medications and antifungal agents in liposomal forms are commercially available.

➢ Classification of Liposomes[4-6]

The Liposomes classification are based on-

i. Structure
ii. Preparation Process
iii. Constituent and its Application
iv. Conventional liposome
v. Liposome specialty

1) Based on Structure

Table 1: Diameter Size and number of lipid layers of different vesicles

Vesicle type	Abbreviation	Diameter Size	No. of Lipid Layers
Unilamellar vesicle	UV	All size ranges	One
Small Unilamellar vesicle	SUV	20-100 nm	One
Medium Unilamellar vesicle	MUV	More than 100 nm	One
Large Unilamellar vesicle	LUV	More than 100 nm	One
Giant Unilamellar vesicle	GUV	More than 1.0 µm	One
Oligolamellar vesicle	OLV	0.1-1.0 µm	Approx. 0.5
Multilamellar vesicle	MLV	More than 0.5 µm	5-25
Multi vesicular vesicle	MV	More than 1.0 µm	Multi compartmental structure

2) Based on Method of Preparation

Table 2: Different methods of preparation and the vesicles developed by those methods

Preparation Method	Vesicle Type
Lamellar vesicle of a single or oligo formed by reverse phase evaporation	REV
Multi lamellar vesicles formed by the method of reverse phase evaporation	MLV-REV
Stable pluri lamellar vesicle	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV
Vesicle prepared by extrusion technique	VET
Dehydration-Rehydration method	DRV

3) Based on Composition and Application

Table 3: Different Liposome with their Compositions

Type of Liposome	Abbreviation	Composition
Conventional	CL	Neutral or negatively charge phospholipids and cholesterol
Fusogenic	RSVE	Reconstituted sendai virus envelops
pH sensitive	-	Phospholipids such as DOPE or PER with either OA or CHEMS
Cationic	-	Cationic lipid with DOPE
Long circulatory	LCL	Neutral high temp, cholesterol, and 5-10% PEG, DSP
Immuno	IL	CL or LCL with monoclonal antibody linked or sequences of recognition

4) Based upon Conventional Liposome

i. Normalize Mixtures of Natural Lecithin (PC)

ii. Glycolipid-loaded liposome

iii. Synthetic phospholipids with the same chain as natural phospholipids

5) Based upon Specialty Liposome

i. Carbohydrate coated

ii. Bipolar fatty acid

iii. Lipoprotein coated

iv. Methyl/ Methylene x- linked

v. Multiple encapsulated

vi. Antibody directed

There are several pathways in and outside the body by which liposomes can operate, which are as follows[7]:-

i. Liposome binds to the plasma membrane and seems to fuse with them, allowing the substance to be released into the cell.

ii. When they are absorbed by the cell, their phospholipids are linked further into plasma membrane, allowing the medication to be released imprisoned inside.

iii. The liposomes are taken up in the case of phagocyte cells, organelles called lysosomes function on the phospholipid walls and the active pharmaceutical ingredients are released.

STRUCTURAL COMPONENTS OF LIPOSOMES

The major constituents of liposome includes

- Phospholipids
- Cholesterol

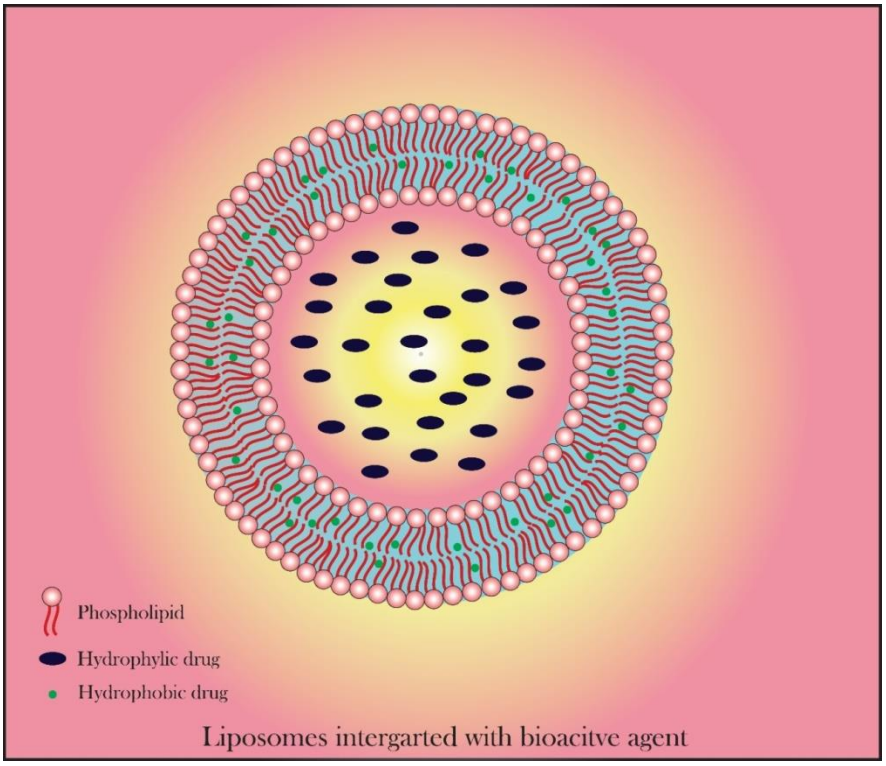
Phospholipids

The fundamental building blocks of bio membranes are phospholipids. Phosphatidylcholine (PC) is perhaps the most often utilised phospholipid in liposome composition.[8] Phosphatidylcholines derived from natural origins or synthesized using semi-synthetic and synthetic techniques. In terms of bilayer sheet orientation in relation to micellar compositions, phosphatidylcholines vary significantly from other phospholipids. The preparation of liposomes is based on a number of natural and semi-synthetic phospholipids.

The molecules of phosphatidylcholine are not miscible in water. They develop a planar bilayer configuration in aqueous environments to decrease undesirable interactions between the bulk aqueous environments as well as in non-esterified fatty acid. The sheets are then folded into enclosed capsules.[9,10]

Cholesterol

Typically, liposomes prepared by using only phospholipids are not sufficiently rigid primarily because of low phase transition temperature and/or unsaturation in the fatty alkyl chains creating defects in the cell membrane like packaging. During packaging those liposome's leak the encapsulated drug. One or two bilayer stabilizers are also used in most liposome formulation in order to avoid such leakage. The additives more widely used are cholesterol and alpha-tocopherol. Liposome encapsulation quality varies with variations in the composition of the phospholipid bilayer. Cholesterol is indeed an absolutely vital component of natural lipid bilayers and its presence in bilayer liposomes induces drastic alteration in their characteristics. Cholesterol by itself doesn't develop bilayer complexes, but can be integrated into high concentrations of phospholipid membranes. Rigidity is enhanced due to compact stacking of the bilayers, and permeability of water-soluble molecules is reduced. By reducing bilayer permeability, cholesterol enhances the durability of hydrophilic drugs. Cholesterol reduces the fluidity above the phase transition temperature (Tc) to make the bilayer more ordered.[11] The tricyclic ring is wedged among the first few carbons of the fatty acyl chains, and the hydroxyl group is exposed to the liquid phase, the cholesterol molecule fits in with the phospholipid molecules and orients itself among them. At very significant concentrations, cholesterol to phospholipids ratios of up to 1:1 or even 2:1 can be incorporated into the cellular membrane.[12] Albumin, macroglobulin, and m-transferrin are blood proteins that interact more easily and frequently with cholesterol-free liposomes enabling the vesicle to become unstable as a result its usage as a therapeutic delivery method has declined.



The figure is showing that the drugs can either be incorporated inside the aqueous space (hydrophilic drugs) or inside the phospholipid bilayer (hydrophobic drugs) of liposomes for the targeted drug delivery.

THE FORMATION OF VESICLES

To synthesize lipid vesicles, thin layer lipid films must be properly hydrated and inflated. During agitation, large MLVs are formed when hydrated lipid sheets split, stopping water from interfering with the bilayer's hydrocarbon core at the margins. After the particles are produced, they have been sonicated or extruded to decrease their size.[11]

METHODS OF PREPARATION

Basically, all the liposome preparation strategies have four basic steps.

I. Drying down lipid from organic solvent

II. Dispersing the lipid in aqueous media

III. The purification of the resulting liposome

IV. Examination of final products

The three distinct liposome preparation methods are as follows:

1) Passive loading technique

a) Mechanical Dispersion

i. Lipid film hydration method (Hand shaking/Non-hand shaking)

ii. Micro emulsification

iii. Sonication

iv. French pressure cell

v. Membrane extrusion

vi. Dried reconstituted vesicles

vii. Freeze-thawed liposomes

b) Solvent dispersion

i. Ethanol injection method

ii. Ether injection method

iii. Double emulsion

iv. Reverse-Phase evaporation

c) Detergent Removal

i. Detergent removal from mixed micelles vesicle by Dialysis dilution

2) Active loading technique

a) Proliposome lyophilization

1. Technique of passive loading [13- 15]

a) Mechanical dispersion

- **Lipid Hydration Method**

This is perhaps the most well-known and widely utilized approach to make MLVs. The round bottomed flask can be utilised for its formulation. The procedure entails forming a thin coating by dehydrating the lipophilic mixture and on hydrating the membrane by introducing an aqueous buffer as well as thoroughly mixed the dispersion. The hydration stage is performed at a temperature higher than the gel–liquid-crystalline (Lβ→Lα) transition temperatures of the lipid or just above the transition temperature of the lipid mixture's maximal melting part. The pharmaceuticals somehow been enclosed are introduced to an aqueous buffer or into an organic solvent comprising lipids solvents depending on their solubilites. The method's drawbacks include low internal volume, lower efficiency of encapsulation and differing size. Diethyl ether overcomes the poor encapsulation ability of lipids by hydrating them in the presence of immiscible organic solvents like petroleum ether. After that, sonication is used to emulsify the mixture. By removing organic layer by passing nitrogen, MLVs are developed.[16]

- **Micro emulsification**

This technique is used in commercial scales to prepare small lipid vesicles. For the preparation of tiny vesicles somewhat from concentrated lipid suspension, an apparatus called a micro fluidizer is used. As a suspension of large MLVs, the lipids should be taken into the fluidizer. This machine pumps the fluid into a 5μm panel at very high pressure.[17] Then a long micro channel is forced, which drives two fluid streams colliding at very high velocity at the right angle together. Micro emulsion can be developed for biological applications by regulating rotation speeds from 20 to 200.[18]

- **Sonication**

This technique decreases the size of the vesicles and provides energy to lipid mixture. This can be accomplished by irradiating the MLV with ultrasonic waves. Sonication can be done in two different ways (i) using bath sonicator (ii) using probe sonicator. The probe sonicator is often used for suspensions that demand a lot of energy in a little amount of volume.[19-21.] For substantial volumes of dilute lipids, the bath sonicator is used. The primary disadvantages of this technique are its poor internal encapsulation efficacy, phospholipid disintegration, elimination of particularly huge molecules, metal impurities from the probe tip, and the coexistence of MLV and SUV.[22,23]

- **French Pressure Cell Method**

MLV is extruded via a tiny hole at 20,000 pressure and 4°C during the process. In comparison to sonication techniques, the method offers numerous advantages. The approach is simple, quick, and repeatable, yet it entails carrying hazardous materials with care. Liposomes produced as a result are considerably bigger than those produced by sonicated SUVs. The drawbacks comprise difficulties in attaining temperature and the working volumes are comparatively smaller (a maximum of 50 mL).[24]

- **Membrane extrusion**

A heterogeneous liposomal suspension is processed via a polymer sieve with a web like fabrication that develops a tortuous-path capillary pore, an interconnected network having not less than 100 micron thick membrane. The liposomes that were treated had a small distribution of size and a chosen mean size of less than about 0.4 microns.[25] Both LUVs and MLVs can be processed using this approach.

- **Dried reconstituted vesicles**

In this approach, liposomes are combined with lyophilized protein or introduced to an aqueous solution containing pharmaceutical and after that dehydrated.[26]

- **Freeze-Thaw Method**

In this procedure, SUVs are rapidly frozen then gradually thawed. Aggregated compounds are dispersed to LUV by sonication. The formation of unilamellar vesicles in the freezing and thawing processes is a result of SUV17. This form of fusion is highly hindered by enhancing the medium's ionic strength and by enhancing the amount of phospholipids. This technique was used to achieve entrapment efficiency of about 20 to 30%.[27]

b) Solvent dispersion

- **Ethanol Injection Method**

This is simple technique. In this approach, an ethanol lipid solution is quickly incorporated directly through a fine needle into an excess of saline or other aqueous media.[28] In water, ethanol is dissolved and phospholipid molecules are uniformly distributed through the medium. The main disadvantage of this approach is that the particles can have a heterogeneous particles size (30-110 nm). Another major downside is that it is difficult to remove all ethanol, which may lead to the formation of azeotrope with water.[22]

- **Double emulsification**

In this approach, a primary emulsion is produced by mixing the therapeutics in an aqueous phase (w_1), which is further emulsified to produce a primary w_1/o emulsion in an organic polymer solvent. The w1/o/w2 double emulsion is created by combining the foremost emulsion with an emulsifier-containing aqueous solution (w2).[23] In the aqueous continuous stage, when the solvent is removed, microspheres are left behind that are further separated by filtering/centrifuging.

- **Reverse-phase evaporation**

In an Erlenmeyer bulb the lipid mixture is taken and the solvent is removed at decreased pressure using a rotary evaporator. The lipids are disintegrated in the organic phase once the nitrogen has been removed.[29] This phase will see the development of reverse phase vesicles. The standard solvents used are isopropyl ether and diethyl ether. Just after the lipids have been re-dispersed among this phase, an aqueous phase containing the pharmaceutical to be encapsulated is added. The all-aqueous systems are sonicated till the combination generates a distinct one-phase dispersion and the system is preserved under nitrogen pressure at all times. The combination is further transferred to arotovap and organic solvent removal is carried out until a gel is developed followed by non-encapsulated substance removal.[29,30.] Reverse-phase evaporation vesicles are the resulting liposomes that emerge from this process. The method's major benefit is its extremely high encapsulation efficiency.[31,32]

c) Detergent removal

At their optimum micellar concentrations, detergents solubilize lipids. The micelles became more enriched in phospholipids when the detergent was eliminated using dialysis and eventually fused to create LUVs.[14] High reproducibility as well as the development of homogenous liposome populations are two of the positive effects of the detergent dialysis approach. The primary downside of the technique is that detergent residues are retained.[15]

2. Active loading technique

- **Proliposome**

In this technique lipid and drug were coated onto a soluble carrier to develop free flowing granular component in pro-liposome which when hydrated forms an isotonic liposomal solution.[33] The pro-liposome strategic approach will offer an incentive for large-scale, cost-effective manufacturing of liposomes incorporating lipophilic drugs in particular.[34]

- **Lyophilization**

The separation of water from substances at highly reduced pressure in the frozen state is termed cryodesiccation (freeze drying). The technique is extensively employed to dry thermolabile materials that can be ruined by heat-drying. With regard to liposomal stability, this procedure serves as an important tool for resolving long-term stability issues. During the freeze-drying and reconstitution process, leakage of trapped materials can take place.[35,36]

SIZING OF LIPOSOME

The size of the liposome seems to have a significant impact on their fate or the purpose for which it may be employed. The functional viability and uniformity of phospholipids bilayer are required for therapeutic uses of liposomes.[37- 39.] Therefore, with particle size distribution within a certain size range, the liposome processing process must be predictable and reproducible. The typical methods of sizing liposomes are sequential extrusion, gel chromatography and sonification.[40]

However, these methods have the following disadvantages in the long run:

1. It's difficult to keep oxygen out, which leads to a per oxidation process.
2. Metal particles are shed by titanium probes, resulting in toxicity.
3. They will develop aerosols that restrict them from the use of such agents.

These issues are primarily due to the probe's sonication, but using the bath sonication will eliminate these problems.

CHARACTERIZATION OF LIPOSOMES [41- 46]

The liposome must be characterized after preparation and before use in immunoassays. The assessment may be categorized into three broad categories: physical, chemical and biological methods. The physical methods require different criteria, such as shape, size, surface features, lamellarity phase behaviors and drug release profile.

Chemical characterization refers to research that have determined the purity and efficiency of different liposomal components.

Biological characterization aids in the analysis of a formulation's effectiveness and viability for in vivo pharmacological intervention.

1. Visual Appearance

The appearance of the liposomal suspension can vary from transparent to milky depending on the particle size and composition. If the turbidity has a bluish shade, the samples are homogeneous; non-liposomal dispersion occurs in a flat, grey color and is most usually a distributed inverse hexagonal phase or dispersed micro crystallites. In addition to contamination of larger particles, an optical microscope can detect liposomes larger than 0.3 μm.

2. Determination of Liposomal Size

The size distribution is of primary importance when liposomes are intended for inhalation or parenteral administration, as it regulates the in vivo fate of liposomes along with the encapsulated drug molecules. Liposome is usually measured by dynamic light scattering. For this method, liposomes with a relatively homogeneous distribution of size are reliable. Gel exclusion chromatography is a direct way of detecting a completely hydrodynamic radius. In the 30-300nm size range, sephacryl-S100 will separate liposomes. SUVs can be isolated from micelles by sepharose columns -4B and -2B.

3. Determination of lamellarity

The determination of lamellarity is important in defining the liposome structure and it's in vivo efficiency. The efficacy of encapsulation and the kinetics of drug release are greatly affected by the amount of lipid bilayers in the liposome. Lamellarity influences the uptake of liposomes and intercellular fate. The Liposomal lamellarity varies greatly depending on lipid selection and methods of preparation. Electron microscopy or spectroscopic techniques may calculate the lamellarity of liposomes. The NMR liposome spectrum is most commonly reported with and without the addition of a paramagnetic agent that changes or bleaches

the signal of the observed nuclei detected on the outer liposome surface.

4. Liposome Stability

Stability is an essential factor to consider when using liposomal drug delivery systems. The processing system and storage conditions have an impact on the physical stability and chemical purity of a drug molecule. The liposomal stability of the drug molecules regulates their therapeutic function. Size, size distribution, composition and drug retention are correlated with the physical integrity of liposomes, whereas Chemical equilibrium struggles with phospholipid oxidation and hydrolysis, as well as drug degradation. A safe liposomal medicinal product has a qualified physical, chemical and microbial stability that guarantees the integrity of the products during its storage time. Therefore, a given stability study protocol emphasizes its significance in deciding the products physical and chemical integrity having a well-established methods for development, characterization, effectiveness and stability testing.

5. Entrapped Volume

This is an important parameter that regulates vesicle morphology. The entrapped liposome volume (in µL/mg phospholipids) can also be deduced from measurements of the cumulative amount of solute trapped within liposomes, ensuring that the concentration of solute within liposomes in the aqueous medium is the same after isolation from the untrapped material. During the drying down process, water from the inner compartment may be lost in two phases of preparation to eliminate organic solvent.

This can be calculated by a given formula

$$\% \text{ Entrapment Efficiency} = \frac{\text{Entrapped Drug} \times 100}{\text{Total Drug}}$$

6. Surface Charge

The nature and density of charge on the liposome surface will regulate the lipid-cell interaction. Liposomes are typically prepared by means of charge imparting/constituting lipids and hence the charge on the surface of the vesicle is examined. In general, two procedures are employed to analyse the charge: free flow electrophoresis and zeta potential testing.

STABILIZATION OF LIPOSOME

Liposome safety should conform with the same quality as conventional drug formulations. The durability of any pharmaceutical substance is the potential of the delivery mechanism to stay within specified or predefined limits over a fixed period in the prescribed formulation. Chemical stabilization necessitates the avoidance of ester bond hydrolysis in phospholipid bilayers as well as the oxidation of unsaturated lipid chain sites. Physical instability or leaking of encapsulated drug from bilayers leads to vesicle aggregation as a result of chemical instability.[47]

Efficient formulation and lyophilization are methods that can be taken to improve liposomal stability. Generally, liposomes can cause stability problems during the storage period. Certain factors need typically be addressed in order to ensure effective formulation of a stable liposomal drug component: [48,49]

I. Processing with fresh, purified lipids and solvents

II. Elevated temperature avoidance and excessive shear forces

III. Low oxygen demand must be maintained (Nitrogen purging)

IV. Use of antioxidant or metal chelators

V. Formulating at neutral pH

VI. Usage of lyo-protectant in freeze-drying conditions

APPLICATION OF LIPOSOME

Liposome research has progressed dramatically in the previous 30 years. A large variety of liposome's of variable sizes, phospholipid constituents, cholesterol constituents and surface morphologies can now be developed suitable for a wide range of applications.[50]

The liver and spleen may be targeted using the liposome carrier, and tomography can easily differentiate between malignant and non - malignant tissue. Liposome has a great use in the case of the transdermal drug delivery system. In the treatment of tumor cells the liposomal drug delivery mechanism contributes to a decrease in the toxic effect and increases drug efficacy. Liposomes are targeted at the region of operation by binding a fragment of amino acid to particular cell receptors.[51]

DNA vaccination and increased gene therapy success is only a couple of the latest liposomal treatment applications. Many sorts of drug administration implementations have been suggested for the liposomal drug delivery, some of which are mentioned below:

I. Enhance drug solubilisation (Cyclosporins, Minoxidil, Amphotericin-B and Paclitaxels)

II. Drug molecules that are sensitive are protected (Cytosine arabinosa, Ribozymes, DNA, Anti-sense oligo-nucleotides and RNA)

III. Enhance intracellular uptake (Anti-microbial, Anti-tumor and anti-viral drugs)

IV. Modification in pharmacokinetics and bio-distribution(prolonged or sustained released drugs with short circulatory half-life)

A. Liposome for Respiratory Drug Delivery System[52]

Liposomes are typically used in many forms of respiratory illnesses. It is possible to formulate liposomal aerosols to develop prolonged release, avoid local inflammation, minimize side effects and improve stability throughout the vast aqueous core.

Numerous injectable liposome-based drugs, including ambisome, Fungisome and Myocet, are now available in the market. To be successful, the delivery mechanism of liposomal drugs to the lungs depends on lipid composition, charge, size, drug & lipid ratio and delivery techniques. The emerging application of liposomes for DNA transmission to the lungs indicating that a better knowledge of their usage in macromolecules administration by inhalation is now developing. All of this new expertise can be used to improve liposome-based protein formulations. The liquid or dried form is utilized for liposome inhalation and the pharmaceutical is released during nebulization. Milling or sprays drying have been used to develop drug powder liposomes.

B. Liposome in Eye Disorders

Liposomes have long been utilised to treat both the anterior and posterior regions of the eye. Dryness, keratitis, proliferative vitreoretinopathy, endopthelmitis, and corneal graft rejection are all eye diseases. In developing nations,

retinal abnormalities constitute the single biggest cause of blindness. Liposomes are employed as a genetic transfection vector and a carrier for monoclonal antibodies. In the therapy of targeted tumors and neovascular artery occlusion, angiography, retinal and choroidal blood vessel stasis, recent treatment strategies such as the application of heat-activated liposomes in focused lasers, as well as heat-induced release of liposomal therapeutics and dyes for targeted delivery. To far, two patent medicines have received approval for liposomal drug formulations, and many more are undergoing scientific testing. 'Verteporfin' is a commercially endorsed liposomal medication for ocular usage. The relevance of the liposome will be expanded in the future to include the therapeutic, diagnostic, and research aspects of ophthalmology.[53]

C. Liposome as Vaccine Adjuvant [54]

Liposomes are a well-known immune adjuvant that enhances both cell-mediated and non-cell-mediated immunity. Liposomal immune adjuvants work by releasing encapsulated antigen slowly and passively into the localized lymph node after intramuscular injection. Accumulation of liposomes into lymphoid is accomplished by targeting liposomes with the aid of phosphotidyl serine. It is possible to prepare a liposomal vaccine by immunizing bacteria, soluble antigen and deoxyribonucleic acid cytokinesis with liposomes. However, it induces immunological reaction to antigenic protein expression. Antigens will further combine with the liposomal membrane in a covalent manner. Liposomal vaccines can be kept for around 12 months in a refrigerated state.[55]

D. Liposomes for Brain Targeting

Liposomes have lately gained popularity as a brain drug delivery mechanism due to their biocompatibility and biodegradability.[56] Liposomes with small (100 nm) or even big diameters diffuse freely across the Blood Brain Barrier (BBB). SUVs linked to cognitive drug carriers, on the other hand can be carried across the BBB by receptor-mediated or absorptive-mediated transcytosis. Absorptive mediated endocytosis of cationic liposomes occurs in cells, however it has yet to be proven that absorptive induced transcytosis occurs across the BBB .The mannose-coated liposomes reach the brain and help in drug transport across the BBB. When systemically administered, the Leu-enkephalin, mefenkephalinkyoforphin and neutropeptides do not typically penetrate the blood-brain barrier. Due to the versatility of this approach, the anti-depressant amitriptyline usually penetrates the BBB. Nanoparticles (NP) have been developed from various stabilizers. The amount of amitriptyline was observed to be substantially increased in the brain when the drug was adsorbed to the NP and wrapped or particle remained stable with polyoxyethylene 20 sorbitan trioleate.[57]

E. Liposome as Anti-Infective Agents

The intracellular pathogen such as protozoa, bacteria and fungi reside in the liver and spleen and thus the therapeutic substances can be administered to such organs using liposomes as the transport system to remove these pathogen.[58] It is possible to treat diseases such as leishmaniasis, histoplasmosis, candidiasis, erythrococosis, aspergelosis, gerardiasis, tuberculosis and malaria by integrating and targeting the medication with the liposomal carrier.

Amphotericin B, a polyene antibiotic is associated with significant kidney damage when used to treat systemic fungal infection. Amphotericin B acts by binding to sterol in the membrane of susceptible fungi, thereby enhancing the permeability of the membrane. Because of its non-specificity and affinity to cholesterol in mammalian cells this substance is hazardous. The first liposome formulation of Amphotericin B has recently been performed in all clinical tests and is now commercialized fortreating various fungal diseases such as Mucormycosis (Black Fungus), aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, and cryptococcosis. Liposomal Amphotericin B decreases renal and general toxicity at the usual dosage by passively targeting the liver and spleen, but renal toxicity usually happens whenever drug is delivered at a high dose due to liver and spleen macrophage saturation. By coating the vesicle using o-stearoyl amylopectin, polyoxyl ethylene, or mono-sialogangliocyte liposomes can also be specifically targeted to the lungs. The encapsulation in the lung-targeted liposome of ant tuberculous agents such as isoniazid and rifampicin modulates toxicity and increases the effectiveness of these products.[59] Varieties of formulations of amphotericin have been approved in many clinical trials and are now being sold in numerous European countries.[60]

F. Liposome in Tumor Cell Therapy

Anti-cancer medicines have serious hazardous side effects when taken for a long time. The liposomal treatment for tumor cell targeting has enhanced the field of tumor treatment by reducing side effects to a minimum. Although the tiny and stable liposome are supposed to be passively targeted to various tumors although they can circulate for prolonged periods of time and can additionally vasate in tissues with increased vascular permeability.[61,62.] The intake of liposome macrophages by the liver and spleen has impeded liposome growth as a drug delivery for more than 20 years. Many herbal anticancer medications have now been incorporated into liposome to provide improved targeting with increased bioavailability.[63]

ADVANCEMENTS IN LIPOSOMES

- **Ethosomes:** They are effective in delivering soya phosphatidylcholine and 30% ethanol to the skin.
- **Immuno liposomes:** They were modified with antibodies.
- **Niosomes:** They are small unilamellar vesicles composed of surfactants that are non-ionic.[64]
- **Stealth liposomes:** These are new types of liposome designed to enhance stability and extend their half-life in circulation. Coating of liposomes should be done by poly ethylene glycol (PEG) for preparing these liposomes.[8]

Liposome's in biomedical research applications: An Experiment

The use of liposomes in healthcare has the potential to provide innovative and effective therapies for a variety of pathological diseases. At the trial in vitro and in vivo stages there appears to be a significant increase in lipid–based therapeutic carrier research. Liposomes are being used to transport a broad variety of therapeutic and diagnostic components including therapeutic molecules, bioactive agents and gene therapy.[65] Alterations in lipid content, charge and the inclusion of surface coatings and ligands are all being looked into to increase effectiveness, reduce RES clearance, and limit toxicity.[65,66]

For a number of biological applications, active targeting methods involving the conjugation of targeting ligands to the surface of liposomes have been widely explored at the early stage of the research especially following parenteral injection.[67- 69.] Targeting ligands are utilised to enhance the selectivity of encapsulated cargo delivery to and retention in

disordered cellular components with even less non-target deposition. Following the accumulation of nano carriers in affected tissues, it's likely that adding targeting moieties boosts receptor-mediated absorption of drug-encapsulated liposomes into target cells ultimately improving therapy efficacy.[70,71]

Despite the fact that ligand-targeted liposomes have enhanced bio distribution and therapeutic results in a majority of preclinical investigations, the positive impacts have been minimal in clinical trials thus far.[72] The optimal density of targeting ligands on the surface of each liposome is yet unclear, and will most likely be determined by the molecular target's properties. We are learning more about the more relevant clinical indications for ligand-targeted liposomal formulations as a result of our comprehensive testing. Furthermore, charged lipids have been used to modify the lipid bilayer, which has gotten a lot of interest.[73] The incorporation of charged lipids into the liposomal bilayer has aided in the development of bioadhesive, mucoadhesive, and nucleic acid-based delivery systems. Using triggering mechanisms for site-specific release of medicines from liposomes is another way to increase therapeutic effectiveness of liposomal formulations. Knowing the accomplishments in liposomal innovation thus far as well as the obstacles that remain, will enable further research to enhance on legacy systems and solve present translational and regulatory restrictions.[74,75]

CONCLUSION

The development of numerous types of liposome's as a targeted drug delivery system for treating various diseases has exploded in the last decade. This review discussed liposomes as a targeted drug delivery method, including their current status, limitations, and future prospects. The versatility of their behavior could also be used to administer drugs by any route and for every drug ingredient regardless of solubility capabilities. Liposome's effectiveness as therapeutic agents has led to the development of a variety of liposome-based formulations that provide greater concentration of drug. The liposomal method can be used to increase pharmacokinetics and therapeutic effect while lowering the toxic effects of a variety of enormously potent therapeutics. Without a doubt the liposomal delivery method has the ability to transform conventional therapy for the cure of a wide range of life-threatening illnesses including cancer. Liposome's usage in pharmaceutical and gene delivery is promising and it will undoubtedly be further developed in near future. The development of liposome's as a drug carrier will remain to be a priority in the coming years with a higher potential to be transferred into the therapy.

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