Review Article

Liposomes as targeted drug delivery system: A review

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ABSTRACT

Liposomes have drawn the attention of various biological researchers which act as potential carriers as well as targeted drug delivery system that would contain various bioactive molecules which have a wide range of therapeutic application. Liposomes are artificial spherical vesicles made up of phospholipids which are non-toxic in nature and cholesterol. They are known promising systems of novel drug delivery since it has a definite size, shape, and hydrophilic and hydrophobic property. Liposomes are made up of lipid body and subcellular components which resemble the structure of ribosomes in which active drug is encapsulated inside the vesicles. Liposome encapsulation is widely used in cosmetic and pharmaceutical industry where liposomes are used as curative promoters in the proposal if targeted drug delivery. They are the submicroscopic foams which act as the biological barrier against the saliva, stomach, alkaline juice of the intestine, bile, intestine flora as well as free radicals and also defended from oxidation and degradation. Phospholipid remains intact until all contents are dispatched to targeted site or organ. The main purpose is to increase therapeutic index and decrease side effects. In this review, the structure, classification, method of preparation, characterization, and applications are discussed below.

KEY WORDS: Bilayer, Carriers, Cell membrane, Cholesterol, Liposomes, Phospholipids, Surfactants

INTRODUCTION

Liposomes are tiny vesicles (bubbles) made up of material which is similar to the cell membrane. Liposomes are filled with drugs, which cures many complicated diseases.[1]

Earlier, liposomes prepared with various classes lipids and remain identical to the biological membrane. In the mid of the 1960s, liposomes were originally discovered and deliberated as the model of cell membrane, and coined by the term "magic bullet" by Paul Ehrlich. This drug loading process was put forth to fetch the drug to the site of action and thereby emancipate drug for the therapeutic activity while the non-target drug as an example of drug effect. Liposomes vesicles made up of concentric bilayer phospholipids from moderate molecular weight to higher molecular which are enveloped as in liposomes along with the drug. Drug constituents are incorporated inside the liposomes and in-taken through oral, nasal, and rectal and also administered through intravenous, ocular, and topical routes of administration. The drugs which dissolve water stay in aqueous chambers while lipid-soluble and amphiphilic compounded drugs attach in phospholipids bilayer. The size range of multilamellar vesicle (MLV) may vary between 500 and 10,000 nm. Large unilamellar vesicle (LUV) is >50 nm and distributed asymmetrically in bilayer phospholipids termed as asymmetric liposomes. Liposomes can be either UV or multilamellar and also known as giant liposomes which have the size range from 10,000 to 1,000,000 nm. Direct delivery of drugs onto the skin in the form of liposomes acts as a fruitful treatment for treating local dermatological complications. Liposomes are the superior most carrier and more efficient to encapsulate both hydrophilic and lipophilic drugs by preventing the degradation of drugs from acidic and alkaline environments (in the stomach). Topical route of drug administration is the most localized route in the body where the drug can be administered through ophthalmic, rectal, vaginal and dermal routes. Here, skin is the major attainable organ and primary path on the human body for topical administration.

Liposomes have been noted for its stability and effectiveness as drug carriers, i.e., for their drug holding capacity. The storage of liposomes was assessed for 5 weeks, under specified conditions. Delivery of liposome to the dermatological region is considered an efficacious treatment for the confined disease (dermatological compilations). The topical liposome is

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prepared by hydration of lipids, which is hydrated from the dry state (spray dried powder) lipid molecules. Then, the crystalline bilayer of this molecule gets fluidized and swells as long myelin thin cylinders grow on upon continuous agitation, which leads to the detachment itself into large multilamellar liposomes. After generating the large particles, with the help of mechanical treatment, liposomes are broken into small bilayered fragments (smaller liposomes). It is very strenuous to calculate the size of liposomes by budding off mechanism.[2]

Liposomes prepared in each core hydro concerning aqueous solution are known as micelles. Reverse micelles are used to circumscribe an aqueous environment.[3] Encapsulated drugs of liposomes obtain the therapeutic level for prolonged action as the drug should be released before it is metabolized and eliminated.[4]

Liposomes protect the drug from the acidic and alkaline attack of saliva, acids of the stomach, alkaline juice of the intestine, bile as well as free radicals and also prevent from oxidation and degradation by acting as a barrier. In general, phospholipids composed of two layers and remain stable where the tails face outside toward and line up together to create a facade off the water in one layer. Here, the head is dragged toward water and tail is offended by water. In the same way, the another layer, heads are brought by water and follow-up to form a surface of the water. Hydrophilic head group and the hydrophobic tail group molecules are made up of phospholipids.[5]

Topical application of liposome vesicles had a vast range of advantages in conventional dosage forms and considered to be more adequate and comparatively less toxic than other pharmaceutical sine because of its structure and bilayer composition. In general, liquid or gel formulations of liposomes usually applied to the skin. Topical application of liposomes in gel-based forms, suitable thickening agents used, is hydrophobic polymers. Liposomes are excellent carriers for topical drug administration and specified to assist in drug transport in between the skin layers. Vesicles of liposomes containing drug are presumed to provide lipidic conditions to offer drug molecules to remain in epidermal layers in the site of action.[6,15] Liposome as a scavenger toxicity of drugs on the body can be prevented by injecting empty liposomes with a transmembrane pH gradient. The vesicles will act by scavenging the drugs that are circulated in the blood. Liposomes are also used for the transformation of DNA into a host cell, and it is called lipofection.[27]

A major potential disadvantage of the liposomal carrier is that, following intravenous injection, it is rapidly intercepted by the fixed macrophages of the liver and spleen. However, the involvement of the RES in vesicle uptake is the basis of the mode of action of several licensed liposome-based products, including vaccines against hepatitis A and influenza.[30]

Solubilization

Liposomes may solubilize lipophilic drugs that would otherwise be difficult to administer intravenously.

Protection

Liposome-encapsulated drugs are inaccessible to metabolizing enzymes; conversely, body components (such as erythrocytes or tissues at the injection site) are not directly exposed to the full dose of the drug.

Duration of Action

Liposomes can prolong drug action by slowly releasing the drug in the body. Directing potential targeting options changes the distribution of the drug through the body.

Internalization

Liposomes are endocytosed or phagocytosed by cells, opening up opportunities to use "liposome dependent drugs." Lipid-based structures (not necessarily liposomes) are also able to bring plasmid material into the cell through the same mechanism (non-viral transfection systems).

Amplification

Liposomes can be used as adjuvant in vaccine formulations.[15]

STRUCTURAL COMPONENTS OF LIPOSOMES

Among the wide variety of new drug delivery systems (DDSs), liposomes seem to have the best potential to accommodate both water and lipid soluble compounds. Liposomes protect the encapsulated drug from metabolic degradation and release the active ingredients slowly and in a controlled manner.[7] The major constituents of liposomes may include:

- Phospholipids
- Cholesterol
- Sphingolipids
- Other components.

Phospholipids

Phospholipids are the principal fundamental element to form biological lamina in liposomes. Phospholipids that contain glycerol are commonly used in the formulation of liposomes and constitute over 50% weight of lipid in the biomembrane and isolated from phosphoric acid. The most common phospholipid used in liposomal preparation is phosphatidylcholine (PC). PC is an amphipathic molecule containing.

- Phosphocholine-hydrophilic polar group
- A bond of glycerol
- hydrophobic acyl
- hydrocarbon chains pair.

PC molecule is water insoluble. They regulate

themselves firmly in an aqueous medium in planar bilayer films by reducing the action of the gross aqueous condition and the long fatty chain, followed by the folding of sheets within themselves to form a completely sealed vesicle. Numerous phospholipids used preparation of liposome are as follows:

- Dipalmitoyl PC
- phosphatidylethanolamine
- phosphatidylethanolamine
- Distearoyl phosphatidylethanolamine
- Dioleoyl phosphatidylethanolamine
- Dilauryl phosphatidylglycerol
- Distearoyl phosphatidylserine.

Cholesterol

Cholesterol in the formulation of liposomes is as follows:

- Sterols incorporation in bilayer liposomes produces prominent changes in the membrane formulation
- Cholesterol does not form a structure of bi-layer by itself. Hence it acts as a fluidity buffer.[8]

Sphingolipids

Backbone is sphingosine or a related base which is known to be an important constituent obtained from plants and animals. In general, head group may differ from primary alcohols (choline) to form the complex form of carbohydrates

- Predominantly used sphingolipids - Sphingomyelin and glycosphingolipids
- Gangliosides - the grayish matter, the minor component used for the production of the liposomes.

Sphingolipid molecules contain residues of sialic acid in the polar head group and more negative charge, and this is to afford a layer of the surface exterior charged group.

Polymeric Materials

Phospholipids are made up of synthetic material such as the diacetylenic group of the hydrocarbon chain, which polymerizes when exposed to UV. This leads to the conversion of polymerized liposomes of elevated permeability to trap the drug molecule.

For example, lipids contain the conjugated group of methacrylate and diene.

Other Substances

- Lipid surfactants are used in the formation of liposomes
- Single chain surfactants mixed with cholesterol are also used in liposome formation
- Lipids which are non-ionic
- Many variations which include poly-glycerol and poly-ethoxylated monoalkyl and dialkyl amphiphiles used manufacturing cosmetic products
- Dicetyl phosphate along with sterylamine
- Mono- and di-fluorocarbon chains prominently stable liposomes
- Vesicles comprised of quaternary ammonium salts.[20]

CLASSIFICATION BASED ON LIPOSOMES [Tables 1-3]

Liposomes are classified by: [16]

1. Structural classification
2. Preparation methods
3. Composition and application - classification.

Advantages of Liposome

It provides passive targeting to the tumor tissue (liposomal doxorubicin).

1. Liposome has an elevated level of efficacy and therapeutic index of drug
2. Liposome provides high resistance on encapsulation
3. The liposome is completely biodegradable and biocompatible
4. Entirely non-toxic and its flexible; non-immunogenic on the systemic and non-systemic route of administrations
5. Liposome has reduced toxicity levels of the encapsulating agent
6. Liposomes assist to depreciate the vulnerability of sensible tissues into highly toxic drugs
7. Site recoil impact and incorporated with both water and lipid soluble drug
8. Flexibility to couple with site-specific ligands to achieve active targeting
9. Offers targeted delivery of drug components
10. Can be administered through various routes.

Disadvantages of Liposome

1. Manufacturing cost is huge
2. Leakage and amalgamation of encapsulated drug/

Table 1: Structural classification

Type of vesicle	Abbreviations	Size in diameter	Lipid bilayer (in No)
Unilamellar	UV	All size ranges	One
Small unilamellar	SUV	20–100 nm	One
Medium unilamellar	MUV	>100 nm	One
Large unilamellar	LUV	>100 nm	One
Oligolamellar	OLV	0.1–1 mm	5
Multilamellar	MLV	>0.5 mm	5–25
Multivesicular	MV	>1 mm	Multiple compartment structure

molecules
3. Phospholipid experiences oxidation and hydrolysis like reaction
4. Extremely very short half-life
5. Moderate solubility and stability
6. Rapid uptake by cells of RES
7. It may be allergic due to liposomal constituents.[1,12,14,20]

METHOD OF PREPARATION

Liposomes of varying sizes and lamellarity are formed spontaneously when lipid components are introduced into an aqueous environment. Due to the hydrophobic force, the amphiphilic constituent molecules cluster into aggregates to minimize the contact between their hydrophobic portions and the surrounding aqueous environment; these aggregates can be organized into liposomes if provided with adequate amount of energy in the form of heating, sonication, or homogenization. However, for liposomal preparations to be acceptable as pharmaceuticals, these liposomes must be processed to fulfill criteria such as a defined size and narrow size distribution, and hence, various manufacturing techniques have been developed to achieve the uniformity of the vesicles.[11] The drug encapsulation method in the liposome development is categorized into two groups which include the passive loading, where drug encapsulation happens formation of vesicle, and the active loading, where the drug is entrapped followed by vesicle formation.

Major two methods involved in the preparation of liposomes

- Passive loading techniques
- Active loading techniques.

Passive Loading Method

Passive loading type is accountable for encapsulation of drug component, in generation liposomes. The internal core is loaded with hydrophilic drugs with random mixing along with thin lipid in buffer media. Preparation of this thin, dry film of lipids mixes with lipophilic drugs in other liposomal components. Dialysis or gel filtration chromatography method is used to separate the drug molecules which are not entrapped in the suspension. Encapsulation efficiency in passive loading depends on the concentration of lipids, liposome size, choice of lipids, and water-soluble mixtures does not combine by the lipid bilayer. Higher encapsulation efficiency is more in large vesicles than the small vesicles. The interaction of drug moiety with the lipid has superior encapsulation rate. Other more steps are initiated to develop encapsulation efficiency in the lipophilic chain. This is to elevate the lipophilic property and partition of the bilayer lipid and also a better choice for loading efficiently in this method.

For example, negative charge nucleotide compound (antisense or siRNA) selection of cationic lipid (positive charge) is to improvise the encapsulation efficiency of increased drug/lipid interaction.

Table 2: Preparation methods

Method of preparation	Vesicle type
Single or oligolamellar vesicles by the reverse-phase evaporation method	REVS
Multilamellar vesicles by reverse-phase evaporation method	MLV-REV
Stable pluri-vesicles	SPLV
Freeze and thawed multilamellar vesicles	FATMLV
Vesicle extrusion process	VET
Dehydration-rehydration process	DRV

Active Loading

Drugs molecules that are weakly acidic and alkaline in nature, is constituted as liposomes by active loading technique. This manner is carried out by the inherent potential generated by the electrochemical gradient, pH, or ion gradients over the lipid bilayer. These pH or ion gradients are advanced in liposomes formation by using specified buffer concentration. Then liposomes of external pH replaced with buffers and this leads in creating the pH gradient over the membrane layer. The loaded drug in liposomes is placed typically at a temperature above the phase transition temperature. This is to encounter the fluidity and dynamic transport across the lipid bilayer. After this, drug fragments interact and get charged in favor of ions and entrapped in liposomal core.[13]

Liposome utilization as drug delivery is spiked with the modest encapsulation procedures. The membranous bilayer is general non-permeable to ions and larger molecules of hydrophobic nature. Ionophores regulate the ion transport, while permeation of hydrophobic molecules was controlled by concentration gradients. The advantages of active loading include the following:

Table 3: Composition and application - classification

Liposomal type	Abbreviation	Composition of lipos
Conventional-liposomes	CL	Neutral of negatively charged phospholipids\cholesterol
Fusogenic liposomes	RSVE	Reconstituted virus envelopes
pH-sensitive liposomes	-	Phospholipids such as PER and DOPE among either CHEMS or QA
Cationic liposomes	-	A cationic lipid including DOPE
Long circulatory liposomes	LCL	Neutral high temperature, cholesterol, and 5–9% PEG and DSP
Immunoliposomes	IL	Attached beside antibodies

- More encapsulation capacity and capacity
- A reduced leakage on encapsulation
- "Bed-side" loading of drugs, thus eradicating the loss of retention of drugs by diffusion or toxic gradients
- Constitutive lipids of flexible, as a drug is filled after the formation of carrier unit
- Nullifying biologically active compounds in formulation step and diminishing hazards
- Membrane pH gradient is developed by various methods depending on the nature of drug to be entrapped.[20]

Passive loading techniques include three different methods:

- Mechanical dispersion method
- Solvent dispersion method
- Detergent removal method (removal of non-encapsulated material).

MECHANICAL DISPERSION METHOD

The following are the types of mechanical dispersion methods:

Sonication

Sonication is the commonly used approach for small LUV development. Usually, MLVs are sonicated with the probe sonicator or the bath sonicator under passive atmospheric pressure. The superior shortcomings of this method are that it reproduces very profound internal volume and has low encapsulation efficacy, there is conspicuous degeneration of phospholipids and compounds to be encapsulated, and there is also the elimination of large molecules followed by metal contamination from the probe point and presence of MLV along with SUV.

Probe sonicator

Sonicator tip is instantly immersed into the liposomal content (dispersion). In probe sonicator, the input of energy is engrossed in lipid dispersion. At the tip sonicator, coupling of energy takes place which leads in local hotness; hence, the vessel of liposomal dispersion is placed on top of water bath or ice bath. The process may exceed up to 1 h, where >5% of the lipids can be re-esterified. In this method, titanium will cast off and entirely contaminate the solution.

Bath sonicator

The liposomal content contained in a cylindrical vessel is rested on top of the bath sonicator. Controlling the temperature is more convenient here, while the contrast to sonication by instantly dispersing uses the point. The sterile vessel protects the material implying sonication, diverse to the probe units below an immobile atmosphere.[26]

French Pressure Cell (Extrusion)

MLVs are extruded through a small orifice and have numerous advantages. Usually, vesicle seems to be entrapped solutes which are entirely longer than small LUV. The method used for vesicle formation is sonication or detergent removal by the delicate handling. The resulting liposomes are preferably generous than sonicated SUVs. The crucial impediments of this method are that it is challenging to attain the high temperature and has comparatively minimal working volumes.

Freeze-thawed Liposomes

In the freeze-thawed method, smaller vesicles are frozen rapidly followed the thawing process. The short-lived sonication scatters and gets aggregated into LUV. The process of LUV formation results in complete fusion of SUVs. IIn this type of synthesis phospholipid concentration repressed well by improving the ionic strength. The percentage efficacies obtained is 20-30%.

SOLVENT DISPERSION METHOD

Ether Injection (Solvent Vaporization)

Lipid solution is dissolved in the ether-methanol mixture or diethyl ether, then gradually injected into an aqueous solution, and further encapsulated at 55°C–65°C beneath the reduced pressure. The constant extraction of ether leads to the origin of liposomes. The major drawback of the routine is heterogeneous mixture formation (70–200 nm).

Ethanol Injection

When the lipid solution of ethanol injected spontaneously to a large excess of the buffer, MLVs appear at once. The drawbacks of the order are as follows: The group is heterogeneous (30–110 nm), liposomes are so dilute, the elimination of all ethanol is challenging because it leads to the formation of an azeotrope with water, and the prospect of the multiple biologically active macromolecules to deactivate in the presence of low-quantity ethanol lamellar liposomes.

Detergent Removal Method (Removal of Non-Encapsulated Material)

Dialysis

Lipids are solubilized by detergents at it is critical micelle concentration, where the detergents get detached and micelles increased, and at the end, it gets combined with LUVs. Finally, the detergents are eliminated by dialysis. LipoPrep (Diachema AG, Switzerland), a monetary tool called which is a variant of the dialysis operation, is used concerning the elimination of detergents and performed in a bags containing large detergent free buffers (equilibrium dialysis).

Gel-permeation chromatography

Size special chromatography is used in decreasing the volume of detergent. The liposomes do not perceive

through the pores of the beads packed in a column. They exude within the spaces between bubble. At reduced flow rates, the liposomes get detached from detergent monomers. Then, the swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids and pretreatment by gel filtration column by lipids using empty liposome suspensions.

Drug loading in liposomes

Drug loading can be attained either passively (i.e., the drug is encapsulated during liposome formation) or actively (i.e., after liposome formation). Hydrophobic drugs, for example, amphotericin B (AmpB) taxol or anamycin, can be directly combined into liposomes during vesicle formation, and the amount of uptake and retention is governed by drug-lipid interactions.

Trapping effectiveness of 100% is often achievable, but this depends on the solubility of the drug in the liposome membrane. Passive encapsulation of water-soluble drugs depends on the ability of liposomes to trap aqueous buffer containing a dissolved drug during vesicle formation. Water-soluble drugs that have protonizable amine functions can be actively entrapped by employing pH gradients, which can result in trapping effectiveness approaching 100%.

Freeze drying

It involves the elimination of water from products in the frozen state at tremendously low pressures. The process is frequently used to dry products that are thermolabile and would be devastated by heat drying. The technique has too much potential as a method to solve long-term stability challenges with admiration for liposomal stability. Studies showed that leakage of entrapped materials might take place during the process of freeze-drying and on reconstitution. Recently, it was revealed that liposomes when freeze-dried in the presence of adequate amounts of trehalose (a carbohydrate commonly found at high concentrations in an organism) retained as much as 100% of their original substances. It shows that trehalose is an excellent cryoprotectant (freeze-protectant) for liposome.

Purification of liposome

Purification of liposome is done by gel filtration chromatography, dialysis, and centrifugation. In dialysis method a hollow fiber cartridge is used. SUVs in normal saline may be isolated by centrifuging at 200,000 g, for 10–20 h. MLVs are isolated by centrifuging at 100,000 g for <1 h.

Stealth liposomes and conventional liposomes

Liposomes (biomembranes) are still foreign objects in the body. In general, liposomes are completely removed from the bloodstream and the resistance difficulties are resolved using synthetic phospholipids coated with polyethene glycol, along with chitin derivatives, and then, it is subjected through freeze-drying, polymerization, and microencapsulation of gangliosides. PEG coating in liposomes reduces the percentage of absorption by macrophages which leads to an elevated levels of liposomes in circulation which results in leakage of liposomes in endothelium. A stealth liposome is a sphere-shaped vesicle with a membrane comprised of phospholipid bilayer used to deliver drugs or genetic material into a cell and composed of naturally derived phospholipids with mixed with colloidal suspension. Stealth liposomes are attained and grown in new drug delivery and controlled release.[19]

CHARACTERIZATION AND EVALUATION OF LIPOSOMES

Liposome should be characterized for visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability. The physical and chemical characters of liposomes are governed by these factors.

Drug Content and Content Uniformity

The gel sample (100 mg) was withdrawn, and drug (fluconazole) content was determined using UV spectrophotometer at 260 nm. Similarly, the content uniformity was determined by analyzing drug concentration in gel taken from 3 to 4 different points from the container. In case of liposomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analyzed using UV spectrophotometer at 260 nm.[24]

Stability Studies

The ability of vesicles to retain the drug (i.e., drug retentive behavior) was assessed by keeping the liposomal suspensions and liposomal gel at two different temperature conditions, i.e., 4–8°C (refrigerator; RF) and 25 ± 2°C (room temperature; RT), for 60 days. Samples were withdrawn periodically and analyzed for the drug content, and particle size for liposomal suspension and drug deposition for liposomal gel in the manner described under entrapment efficiency and particle size distribution studies.[28]

A. Biological characterization
   - Sterility - Aerobic/anaerobic culture
   - Pyrogenicity - Temperature (rabbit) response
   - Animal toxicity - Monitoring survival of animals (rats).

B. Chemical characterization
   - Phospholipids concentration - high-performance liquid chromatography (HPLC)/Barrlet assay
   - Cholesterol concentration - HPLC/cholesterol oxide assay
   - Drug concentration - Assay method
   - Phospholipids peroxidation - UV observance

- Phospholipids hydrolysis - HPLC/thin-layer chromatography (TLC)
- Cholesterol autoxidation - HPLC/TLC
- Antioxidant degradation - HPLC/TLC
- pH - PH meter
- Osmolarity - Osmometer.

C. Physical characterization
- Vesicle shape and surface morphology - scanning electron microscope/transmission electron microscopy (TEM)
- Vesicle size and size distribution - dynamic light scattering, TEM
- Surface charge - free flow electrophoresis
- Electrical surface potential and pH - zeta potential and pH-sensitive probes
- Lamellarity - nuclear magnetic resonance (NMR)
- Phase behavior - DSC, freeze-fracture electron microscopy
- Percentage capture - Mini column centrifugation and gel exclusion
- Drug release - Diffuse cell and dialysis.[22,23]

Visual appearance

Based on the particle size and composition, the appearance of the liposomal suspension may be varying from translucent to milky. The samples are homogeneous if the turbidity has a bluish shade; the presence of a non-liposomal dispersion is flat and gray color and is most likely disperse inverse hexagonal phase or dispersed microcrystallites. An optical microscope can detect liposome of size >0.3 μm as well as contamination with larger particles.

Determination of liposomal size

Size distribution

It is usually measured by dynamic light scattering. Liposomes with relatively comparable size distribution are reliable for this method. Gel exclusion chromatography is a simple method, in which a truly hydrodynamic radius can be detected. Sephacryl-S100 can separate liposome in size range of 30–300 nm. Sepharose-4B and -2B columns can separate SUV from micelles.

Determination of lamellarity

The lamellarity of liposomes can be measured by electron microscopy or spectroscopic techniques. The NMR spectrum of the liposome is recorded most frequently with and without the addition of a paramagnetic agent that shifts or bleaches the signal of the observed nuclei on the outer surface of the liposome.

The lamellarity determination is essential to define the structure of liposome and it's *in vivo* performance. The encapsulation efficiency and drug release kinetics are significantly influenced by the number of lipid bilayers of the liposome. The liposome uptake and intercellular fate are affected by the lamellarity. The liposomal lamellarity widely varies based on the choice of lipids and preparation methods.

Liposome stability

Liposome should be physically, chemically, and biologically stable. Physical stability indicates the ratio of lipid to therapeutic agent and steadiness of the size. The chemical stability may be affected by two degradation pathways: Oxidative and hydrolytic. Oxidation of phospholipids in liposomes mainly takes place in unsaturated fatty acyl chain-carrying phospholipids. These chains are oxidized in the absence of particular oxidants. Reduction of oxidation can be achieved by storage at low temperatures and protection from light and oxygen.

Entrapped volume

The entrapped volume of the liposome (in μL/mg phospholipids) can often be deduced from measurements of the total quantity of solute trapped inside liposome, assuring that the concentration of solute in the aqueous medium inside liposomes is the same after separation from the unentrapped material.

Surface charge

Liposomes are usually prepared using charge imparting/constituting lipids, and hence, it is imparting to study the charge on the vesicle surface. The two methods used in general to estimate the charge are free to flow electrophoresis and zeta potential measurement.[8]

Microscopic technique

Optical microscopy

The microscopic method includes the use of Bright Field, Phase Contrast Microscope and Fluorescent Microscope and is useful in evaluating vesicle size of large vesicles.

Cryogenic-TEM techniques

This system has been used to elucidate the surface morphology and size of vesicles.

Diffraction and scattering techniques

Laser light scattering photon correlation spectroscopy is the analysis of time dependence of intensity fluctuation in scattered laser light due to the Brownian motion of particles in solution/suspension. Since small particles disperse more rapidly than large particles, the rate of fluctuation of scattered light intensity varies accordingly. Thus, the translational diffusion coefficient (D) can be estimated, which in turn can be used to determine the mean hydrodynamic radius (Rh) of particles using the Stokes-Einstein equation. Using this technique, one can measure particles in a range of about 3 nm.

In vitro release

A reproducible *in vitro* release method with suitable simulated physiological medium or human plasma should be established for evaluating the release of drug from the liposomes. This method is primarily important for measuring the (a) liposomal drug product quality,(b) suitability of the process controls,(c) drug release from the product over time, and (d) effect of minor changes in the manufacturing process or facility.[11]

Zeta potential

Zeta potential is also known as anionic liposomes and considered to be one of the important factors affecting cellular uptake and drug delivery. Neutral-charged liposomes with tightly packed membranes tend to remain longer in the circulation and exhibit increased drug retention, compared to charged systems. Plasma proteins have an more affinity liposomes and are charged rapidly, and cationic systems are quickly interact with components in the systemic circulation, resulting in a precise half-life *in vivo*.[18]

STABILIZATION OF LIPOSOME

Usually, liposomes may create a problem of instability during the storage period. In general, certain parameters should be considered to achieve successful formulation of stable liposomal drug product:

- Processing with fresh, purified lipids and solvents
- Avoidance of high temperature and excessive shearing stress
- Maintenance of low oxygen potential
- Use of antioxidant or metal chelators
- Formulating at neutral pH
- Use of lyoprotectant when freeze drying.[2]

APPLICATIONS OF LIPOSOMES

Cationic Liposomes for Delivery of Nucleic Acid

Cationic lipids, which are the core components of nanoparticles, have a common structure of a positively charged head group and one or two hydrophobic tail region(s) made of hydrocarbon chains or steroid structures. Lipoplexes of cationic liposomes and nucleic acids still suffer from several limitations. One is their low stability in the bloodstream, which is caused by the characteristics of cationic lipids. Until they reach their target cells, cationic lipid components can interact with serum proteins, potentially disrupting the integrity of liposomal structures or forming aggregates that are too large to be taken up by cells. To increase the *in vivo* stability of lipoplexes in the blood, researchers often include PEG-conjugated lipids and cholesterol as components of the cationic liposomes. Another limitation is the relatively weak delivery of nucleic acids into target cells. For anticancer therapy, enhanced retention and permeability may serve as an initial driving force for the delivery of lipoplexes to tumor tissues. Once in the tumor tissue, the effective recognition of tumor cells and intracellular delivery should proceed.[9,12]

Cisplatin is another widely used anticancer drug, which induces DNA lesions and mitochondrial apoptosis. This, sometimes, may lead to toxicities including nephrotoxicity, neurotoxicity, and ototoxicity which demand new formulations to be developed to reduce toxicity and potentiate efficacy such as cisplatin liposomes.[10]

Liposomes in Cancer

A significant challenge in the treatment of cancer involving chemotherapy is the efficient delivery of cytotoxic agents to tumor tissue while at the same time minimizes the undesired negative side effects associated with these drugs. The use of DDSs such as liposomes can modify drug pharmacokinetics and biodistribution in a manner that improves the overall pharmacological properties of generally used chemotherapeutics. Liposomes are particularly attractive DDS in part due to the ease with which they can be generated and modified such that they can be used to treat a wide variety of cancers. Breast cancer, in particular, has been the focus of many studies concerning liposome-based chemotherapeutics in part due to the clinical success of various drugs such as Doxil, which is a liposomal formulation currently employed to treat recurrent breast cancer.[32]

Sustained Release Liposomes

Anticancer drugs can be delivered to the systemic circulation in a sustained release mode by encapsulating in the liposomes. DepoFoam™, a sustained release injectable technology of Skye Pharma, is applied in DepoCyt® that is used in the treatment of lymphoma, i.e., lymphomatous meningitis. In comparison to unentrapped Cytarabine, DepoCyt® administered through intrathecal route maximized the therapeutic potential of cytotoxic agents that are specific to the S-phase of cell cycle. Furthermore, the dosing frequency reduced due to the prolonged CSF t1/2 of cytarabine.[18]

Liposome Vaccine

Typically, either a purified antigen or an attenuated pathogen is used as an immunogen in a known vaccine. However, a long-term immune response may not be induced by purified antigens and even sometimes does not induce a response at all. On the other hand, attenuated vaccines can produce a reaction in the patient under immunization. Studies revealed that the cell membrane of malignant cells could form liposomes encapsulating potential antigens.[18]

Liposomes for Fungal Treatment

A polyene antibiotic, AmpB, is used for the treatment of fungal infections. It binds to sterols in fungal membrane

as well as mammalian membranes which result in the formation of transmembrane pores where leakage of vital intracellular components takes place leads to cell death. Binding to the cholesterol-containing mammalian membranes results in toxicity. Nephrotoxicity was reported in the systemic use of AmpB and often resulted in central nervous system side effects on chronic use. Studies revealed the effectiveness of liposomal AmpB in experimental fungi and parasitic diseases.[18]

Immunoliposomes

Among the various types of liposomes, immune liposomes have gained wide attention due to their targeting capabilities. Due to the presence of antibodies attached on to their surface, these liposomes exhibit immunologic response. The preparation of immune liposomes, i.e. conjugation of antibodies to liposomes, is not that straightforward and even can pose a challenge during their formulation. Protein molecules and monoclonal antibodies can be conjugated directly on to the liposome, PEGylated liposome, or PEG chain of the PEGylated liposomes. Similar to other liposomes, the RES can scavenge and clear the immunoliposome from systemic circulation. Increased circulation half-life, targeting specificity, and minimized drug loss and degradation are the major advantages of immunoliposomes. Apart from the promising applications, immunoliposomes suffer from a major drawback, i.e., immunogenicity and increased rate of clearance from circulation can be observed due to repeated injections.

Liposomes for Gene Delivery

Liposomes have been extensively studied in areas such as gene therapy and drug delivery due to their observed stability and favorable toxicity profile over traditional treatments. Liposomes can encapsulate biomolecules or drugs that are hydrophilic and increase their internalization and solubility through the lipid bilayers of the cells.[17] Various discoveries related to human genomes and their use in disease treatment have become more approachable with the advances in science and technology. In spite of these developments, choosing a right carrier for the delivery of the gene to the target is of paramount importance. One such important carrier is liposomes, which can deliver DNA, antisense oligonucleotides, siRNA, and other potential agents into the nucleus. Especially, engineered liposomes such as cationic liposomes, pH-sensitive liposomes, fusogenic liposomes, and genomes are explored for gene delivery. The size of the liposomes is an important determinant for clearance by the RES. In this respect, the passage of the endothelium is the first barrier when liposomes are used for gene delivery. The most important target organ for gene transfer is the liver.

Liposomes for Protein and Peptide Delivery

Proteins and peptides are potent therapeutic agents used in the treatment of various diseases. However, due to their unstable nature and degradation at physiological conditions, the delivery of these drugs at the targeted site is extremely complicated. Most of the protein and peptide drugs produce their mechanism of action extracellularly by interacting with the receptors. Encapsulation of proteins and peptides into lipid vesicles for improving the therapeutic properties has been extensively investigated.[32]

Liposomal Inhales

The pulmonary route is a promising drug delivery route. The carriers used for this purpose are target selective and can control drug release. The advantages of the carrier system include decreased drug toxicity, increased the stability of the drug, and the local irritation which is prevented. Nebulizers are used in the actual liposome formulation for inhalation. The nebulizers used include ultrasonic, air-jet, and passively vibrating mesh nebulizers. The drug models with different molecular weights were used. Hydration method was used to prepare liposomes loaded with water-soluble drug. Characterization of liposome was done by an extrusion method, which consisted of an extruder with 100-nm filter of polycarbonate. Various nebulizers were studied to find which one would efficiently atomize the liposome preparation.

Epilepsy

Gossypin liposomes have been prepared in a research study. They studied the efficacy of liposomal gossypin formulations by administering the formulation at two doses by the intravenous route. The studies were carried out in mice treated with pentylenetetrazole to induce seizures. They reported that the increased levels of malondialdehyde and glutathione were reduced and liposomal gossypin suppressed the progression of kindling in mice. These results suggest that liposomal gossypin appears to possess protective activity against kindling in mice.[32]

New Generation of Liposomes

Liposomal drug delivery has created an opportunity to formulate a wide variety of difficult to deliver therapeutic agents. In spite of many products in the market and several others in the clinical trials, the instability of a drug during its transfer to the targeted site is still a problem. Therefore, to improve the drug stability and efficacy and to reduce the adverse effects by targeting the site of action, a new generation of liposomes has been explored using various phospholipids and their derivatives. The new generation liposomes demonstrated considerable advantages with potential therapeutic benefits. However, still, further investigation is needed to overcome the limitations encountered regarding long-term stability, entrapment efficiency, and active targeting.[18]

CONCLUSION

Liposome well-known carrier vesicles also are known for its potential application and acceptable DDS where the drugs are not lessened. The drugs formulated as liposomes are given by numerous ways (intravenous, oral inhalation, local application, and ocular), and these can be used for the execution of multiple disorders. Unusual risk affiliated with drug molecule mentioned as bioavailability, degradation, stability, and side effect can be overwhelmed by consolidating it into the liposome. As it is a unique transport system liposomes provide controlled and sustained release of drugs. The development of deformable liposomes and ethosomes along with the administration of drug through inhalation and ocular route are some of the advances in the technology. Thus, liposomal strategy can be favorably employed to enhance the pharmacokinetics and therapeutic potency, simultaneously diminishing the toxicity of multiple highly potent drugs. Nevertheless, the peculiarity of liposome based on the pharmaceutical applications and available products says that liposomes have not clearly but absolutely most acquire space in pharmaceutical industries and also established their position in modern delivery systems.

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