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(Review Article)

Liposomal carriers: Development, evaluation, applications with its regulatory aspects

THIRUMAL V, ARAVINDHAN V, and NIRANJANASREE AC *

Department of Pharmaceutics, Sri Ramachandra Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, India.

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Abstract

Liposomes are the initially introduced nano targeted drug delivery systems that have been successfully transferred and commercialized due to its high therapeutic index by its clinical applications. Liposomes are colloidal spheres that contain cholesterol, non-toxic surfactants, sphingolipids, glycolipids, long-chain fatty acids, and even membrane proteins and medicinal compounds. These are the most often employed drug delivery vehicles due to their size and amphiphilic characteristics. Drugs that are therapeutically active can be delivered easily using liposomes, including tiny compounds and macromolecules like proteins, peptides, and genes. These range from 25-1000 nm. It used for the administration of various types of drugs and the active substance to treat various diseases by targeting the site. It involves the mechanism of targeting the site without affecting the other cells that is majorly useful in the treatment of cancer. The major advantages of the liposome application are the protection of active compounds from degradation; the increase in circulation time and the possibility to achieve partial or total selectivity. The regulatory aspects are developed over time are described, followed by the lipid family, vesicular drug carrier systems. This review discusses briefly about liposomes and its advantages, disadvantages, types, methods of preparation with its evaluation and the regulatory aspects of the new drug and generic drugs.

Keywords: Liposome; Cholesterol; Amphiphilic; Target; Drug Delivery; Preparation; Evaluation; Regulatory Aspects

1. Introduction

In 1906, Paul Ehrlich initially developed the targeted drug delivery which targets the drug towards the diseased cells termed ‘magic bullets’(1). The name liposome is derived from the Greek words 'lipo' (fat) and 'soma' (body)(2). Bangham et al. discovered liposomes by accident in 1961 by scattering phosphatidylcholine in water, in which bilayer formation occurs with aqueous phase and is surrounded by lipid bilayer(3). It structurally consists of a vesicular, colloidal and comprises of one or more lipid bilayers that surrounds the equal number of aqueous layers(2). These are small and spherical shaped vesicles that are made from non-toxic surfactants, cholesterols, sphingolipids, membrane proteins, glycolipids, and long-chain fatty acids. The phospholipids continuously forms lipid bilayer which is completely closed and surrounds the internal aqueous compartment, which facilitates the transfer of both hydrophilic and lipophilic drugs to the target site(1). The shell which is sphere shaped encapsulates the liquid compartment that can carry substances like hormones, anticancer agents, antifungal, enzymes, antibiotic, proteins, and peptides. When a drug directly injected to blood stream which gives therapeutic level for shorter half-life because of direct distribution and elimination, the drug which carried through the liposomes attains the therapeutic level for longer half-life because the drug should come out from liposome for distribution and elimination(4).

* Corresponding author: NIRANJANASREE AC.

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1.1. Advantages and disadvantages of liposomes:(5), (6)

Table 1 Advantages and disadvantages of liposomes

Advantages	Disadvantages
Amphiphilic More drug loading High therapeutic efficacy More stability due to its encapsulation Reduced toxicity Biocompatible Biodegradable Achieves targeting by coupling with site-specific ligand. Provides sustained release	Less solubility Shorter half-life High cost for formulation Rarely phospholipids undergo hydrolysis or oxidation. Many drawbacks in large scale production.

1.2. Classification of liposomes

1.2.1. Based on the structure parameters(7)

- SUV- Small Unilamellar vesicles (20-100 nm)
- MUV- Medium sized Unilamellar vesicles
- LUV- large Unilamellar vesicles >100
- MLV- Multilamellar large vesicles->0.5 mm
- OLV- Oligolamellar vesicles-0.1-1 mm
- UV- Unilamellar vesicles (All size ranges)
- GUV- Giant Unilamellar vesicles->1 mm
- MV-Multivesicular vesicles->1 mm

1.2.2. Based on method of liposome preparation(7)

- REV- Single or Oligo lamellar vesicle by reverse- phase evaporation method
- MLV-REV- Multilamellar vesicles made by reverse- phase evaporation method
- SPLV- Stable plurilamellar vesicles
- FATMLV- Frozen and thawed MLV
- VET- Vesicles prepared by extrusion technique
- DRV- Dehydration –rehydration method

1.2.3. Based upon composition and application

Liposomes are classified according to their lipid compositions and intended applications in addition to their size and lamellarity(6).

Conventional liposomes

Liposomes are small, spherical artificial vesicles made from cholesterol and natural, harmless phospholipids. Liposomes are particularly attractive drug delivery vehicles due to their small size and amphipathic characteristics (combined with biocompatibility). Liposome properties vary widely depending on lipid composition, surface charge, size, and production method. Furthermore, the choice of bilayer components influences the 'rigidity' and 'fluidity' of the bilayer, as well as its charge(8).

Charged liposomes

Charged liposomes are classified as cationic and anionic liposomes based on their charge density.

Cationic liposomes: The production of cationic liposomes is simple and nonimmunogenic. Many negatively charged DNA, RNA, and oligonucleotides can be transported using cationic liposomes. Additionally, useful for delivering genes

and vaccines into cells. N- [1-(2,3-dioleoloxy) propyl]-N, N, N-trimethylammonium (DOTMA) or N- [1-(2,3-dioleoyloxy) propyl] (DOTAP) are the two phospholipids that are most frequently utilized to make cationic liposomes. The negatively charged nucleotides in these liposomes are neutralized by the cationic components, which are electrostatically associated with them. Thus, nucleotides condense into lipid-nucleotide complexes (lipoplexes), a more compact structure. Compared to DNA encapsulated within the liposome, these lipoplexes provide improved cellular uptake and superior protection(6).

Figure 1 Classification of liposomes based on composition and application

Anionic liposomes: The clearance rate of anionic liposomes was higher than that of neutral and cationic ones. After becoming opsonized with their counterpart and plasma proteins, these liposomes interact with the biological system. Phosphatidyl glycerol, phosphatidic acid, and cardiolipin are a few examples of anionic phospholipids that are frequently utilized to make anionic liposomes. Due to their enhanced skin dispersion properties, anionic liposomes are regarded as excellent carriers for transdermal drug administration(6).

Stealth liposomes

Stealth liposomes are spherical vesicles with a phospholipid bilayer membrane that are used to deliver drugs and genetic material into the circulation. Additionally, these liposomes formulation includes polyethylene glycol (PEG), polyaniline (PA), polyacrylamide (PAA), polyvinylpyrrolidone (PVPA), and others. These polymers were utilized to encapsulate the liposomes or to create their outer membrane(9)

Proliposomes

Dry, free-flowing particles known as proliposomes are described as forming a liposomal dispersion right away when they come in contact with water(10).

Stimuli responsive liposomes

The approach of stimulus-responsive dePEGylation for triggered release is appealing. The release of encapsulated medications to the diseased/target locations may be hampered by the stealth liposomes, despite the fact they have a lengthy circulation period and little payload leakage (PEG dilemma). PEG- (stimuli-responsive linker)-lipid conjugates can be used to cleave PEG (dePEGylate) at the desired places, facilitating drug release at the targeted site or improving internalization of the nanocarriers into target cells. These linkers include enzyme-responsive peptides as well as reducing agent-responsive disulfide and pH-responsive vinyl ether, hydrazone, or hydrazide-hydrazone. The dePEGylation method not only encourages extracellular/intercellular release but also exposes the liposomal surface, which may result in improved liposome-cellular interaction, restored fusogenicity of liposomes, and enabled endosomal escape in addition to a decreased anti-PEG immune response(11).

Bubble liposomes

The term "bubble liposomes" refers to novel varieties of liposomes that contain medicinal payloads and gases. When Maruyama and his colleagues created PEGylated liposomes loaded with plasmid DNA and the ultrasonic imaging gas perfluoropropane, the bubble liposomes were first discovered. Numerous studies have demonstrated the effectiveness of using bubble liposomes to deliver nucleic acid-based therapies both *in vitro* and *in vivo*. Nucleic acids can be electrostatically functionalized to the surface of this type of liposome or trapped inside the liposome's core(6).

1.3. Components of liposomes

1.3.1. Phospholipids

The most often utilised component of liposome formulations, phospholipids that contain glycerol, account for more than 50% of the weight of lipid in biological membranes. These are produced using phosphoric acid. The glycerol moiety serves as the molecule's backbone(1). Phosphatidylcholine is the most popular type of phospholipid utilised in liposomal preparation. An amphipathic compound called phosphatidyl choline contains:

- Phosphocholine is a polar head group that is hydrophilic.
- A pair of hydrophobic acyl hydrocarbon chains with a glycerol bridge(3).

The arrangement of the lipid molecules' hydrocarbon chains affects the stability of the liposome membrane. The kind of fatty acid in the lipid molecule, such as the number of double bonds in the chain, influences bilayer features such as elasticity and phase behaviour. In nature, phospholipids are widely distributed, and choline-containing phospholipids are employed to make liposomes(4). Phosphatidylcholine molecules are insoluble in water. In aqueous conditions, they form closely packed planar bilayer sheets to avoid unfavourable interactions between the long hydrocarbon fatty chain and the bulk aqueous phase. After then, the sheets fold back on themselves to form sealed, closed vesicles(12).

1.3.2. Cholesterol

Another crucial part of the liposome's structural makeup is cholesterol. It is a frequently employed sterol. Sterols are added, which modifies the function of stability and rigidity and lengthens the period that blood is in circulation. It cannot create a bilayer structure on its own(4). It also helps

- By lowering the membrane's permeability to a water-soluble molecule.
- Stabilising the membrane in the presence of biological fluids like plasma
- Decreasing the fluidity or micro viscosity of the bilayer(13).

Examples of phospholipids are

- Phosphatidyl choline (Lecithin) – PC
- Phosphatidyl ethanolamine (Cephalin) – PE
- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG)

Use of saturated fatty acids results in stable liposomes. In general, unsaturated fatty acids are not used(4). An extremely high concentration of it, up to a 1:1 or 2:1 molar ratio of cholesterol to phosphatidyl choline, is integrated into phospholipids. being present cholesterol increases stability and helps to create a stiff, highly organised membrane structure in the lipid bilayer(1).

1.3.3. Sphingolipids

One of the most crucial components of sphingolipids is sphingosine. Cells from both plants and animals can be used to make sphingolipids. For instance, glycosphingolipids and sphingomyelin(2). These are crucial parts of both plant and animal cells. This consists of three defining building blocks.

- A mole of fatty acid
- A mole of sphingosine
- A head group that can range from extremely simple carbohydrates to simple alcohols like choline.

This molecule has one or more negative charges at neutral pH because it contains complex saccharides with one or more salicylic acid residues in its polar head groups. These are incorporated into liposomes to add a layer of charged group to the surface(4).

1.3.4. Cationic lipids

Examples include DOTAP, DOTMA, and cationic compounds of cholesterol as well as numerous analogues of DODAB/C, dioctadecyl dimethyl ammonium bromide or chloride, and dioleoyl propyl trimethyl ammonium chloride(4).

1.3.5. Polymeric materials

When exposed to ultraviolet light, synthetic phospholipids with a diacetylene group in the hydrocarbon chain polymerize, creating polymerized liposomes with noticeably higher permeability barriers to entrapped aqueous pharmaceuticals. For instance, alternative lipids that can be polymerized include those that contain conjugated dienes, methacrylate, etc. Additionally, a number of polymerizable surfactants are created(4).

1.3.6. Polymer bearing lipids

Electrostatic forces that repel one another are primarily responsible for the stability of repulsive interactions with macromolecules. By applying charged polymers to the liposome surfaces, this repelling force can be produced.

Higher solubility is provided by non-ionic and water-compatible polymers such polyethylene oxide, polyvinyl alcohol, and polyoxazolidines. However, adsorption of such copolymers that combine hydrophilic and hydrophobic segments results in liposome leakage, therefore the best outcomes can be obtained by covalently bonding polymers to phospholipids. As an illustration, consider diacyl phosphatidyl ethanolamine and PEG polymer connected by a succinate or carbon at bond(4).

Other substances

- To create liposomes, a range of different lipids and surfactants are utilised.
- Liposomes are created by combining a variety of single-chain surfactants with cholesterol.
- Non-ionic Lipids(2).

2. Mechanism of action of liposomes

Initially thin lipid films are hydrated and swollen for the formation of lipid vesicles. During agitation, the hydrated lipid sheets separate into huge MLV, which prevents water from interacting with the margins of the bilayer's hydrocarbon core. Once created, the particles are extruded or sonicated to minimise their size(1).

The hydrophobic portion is made up of two fatty acid chains with 10–24 carbons, the hydrophilic part is predominantly phosphoric acid linked to a water-soluble molecule. Each chain has atoms and 0-6 double bonds. When dispersed in aquatic fluids, they orient themselves so that the polar head group faces the aqueous region and the fatty acid groups face each other, resulting in liposomes, which are spherical, vesicle-like structures. When energy is added to hydrate phospholipids in water, such as through sonication, shaking, heating, homogenization, etc. Hydrophilic/hydrophobic interactions between lipid-lipid and lipid-water molecules create bilayer vesicles to attain thermodynamic equilibrium in the aqueous phase. As the primary constituents of the cell membrane, phospholipids have great biocompatibility and amphiphilic characteristics(14).

The four different mechanisms by which liposomes work are as follows: -

1. Endocytosis: This process is carried out by phagocytic reticuloendothelial system cells like neutrophils.

2. Adsorption: It takes place on the cell surface as a result of interactions with cell surface elements or non-specific electrostatic forces.

3. Fusion: This process involves the continual release of liposomal content into the cytoplasm as a liposomal bilayer is inserted into the plasma membrane.

4. Lipid exchange: In this process, lipids from liposomes are transferred to the cell membrane without the associated liposomal components(3).

Compared to unbound drug molecules, liposomes show distinct biodistribution and pharmacokinetics. This has several applications for enhancing the therapeutic effectiveness of pharmacological molecules that have been encapsulated. Reduced medicine bioavailability, lipid saturation immune system cells, and maybe increased toxicity of pharmaceuticals due to higher interactions with specific cells are all limitations. The benefits of drug-loaded liposomes, which can be applied as an aerosol, creams, gels, or colloidal solution, can be divided into seven categories:

- Increased medication solubility in lipophilic and amphiphilic environments.
- Passive targeting of immune system cells, particularly mononuclear phagocytic system cells
- A mechanism for the sustained release of liposomes that have been given orally or topically.
- Liposomes have a site-avoidance mechanism that prevents them from accumulating in certain organs like the heart, kidneys, brain, and nervous system, which lowers cardio, nephro and neuro toxicity.
- Site-specific targeting: In some cases, liposomes with surface-attached ligands can connect to target cells [a "key and lock" method] or can be transported into the target tissue by local anatomical features such as leaky and improperly formed blood vessels, their basal lamina, and capillaries.
- Better transfer of charged, hydrophilic compounds like chelators and medicines
- Increased tissue penetration, particularly in case of dermal(10).

2.1. Transportation via liposome mechanism

There are four different ways that liposomes might interact with cells.

- Endocytosis by phagocytic reticuloendothelial system cells like neutrophils and macrophages.
- Either by non-specific, weak hydrophobic or electrostatic forces adhering to the cell surface or by interactions with cell-surface elements.
- Insertion of the liposome's lipid bilayer into the plasma cell membrane and simultaneous release of the liposomal content into the cytoplasm results in fusion with the plasma cell membrane.
- Lipids from liposomes can be transferred to cellular or subcellular membranes or vice versa without the contents of the liposomes being associated(15).

2.2. Liposome characteristics for drug delivery system

- One potential in the pharmaceutical sectors is the use of liposomes as drug delivery systems.
- Some physical and chemical aspects of liposome as a drug delivery mechanism have a dramatic impact on the qualities of nano-medicines, such as release from dosage forms at specified areas as well as drug circulation and absorption into body membranes.
- Zeta potential, also known as electro kinetic potential in colloidal systems, has a significant impact on the many characteristics of liposomes.
- Poor solubility and stability are just two of the problems facing medication delivery systems.
- Two significant variables that may be important in this context are particle size and charge(16)

3. Methods of preparation

Different synthesis methods can be used to create liposomes. The method used to make the liposomes and the kind of phospholipids used have an impact on the intended liposome's charge, size, PDI, and lamellarity(6). The many ways to prepare liposomes fall into the following categories:

- Passive loading techniques
- Active loading techniques.
- The three main ways are included in passive loading:
- Mechanical dispersion method.
- Solvent dispersion method.
- Detergent removal method

3.1. Mechanical dispersion method:

- Sonication method.
- French pressure cell.
- Freeze-thawed method.
- Lipid film hydration method (hand shaking).

- Micro-emulsification method.
- Membrane extrusion method.
- Dried reconstituted vesicles(5).

3.2. Solvent dispersion method:

- Ether injection method.
- Ethanol Injection method.
- Reverse Phase Evaporation techniques.
- Double emulsion method.

3.1. Mechanical dispersion method:

The most typical and popular way to generate multilamellar vesicles (MLV) is this method. For the preparation, a round bottom flask might be utilized. The process comprises of drying the lipid solution to produce a thin film, followed by hydration of the film through the addition of an aqueous buffer then vortexing the dispersion. The hydration process is carried out at a temperature greater than the lipid's gel-liquid-crystalline transition temperature or greater than the transition temperature of the lipid mixture's greatest melting point. The molecules to be encapsulated are added to either an organic solvent containing lipids or an aqueous buffer, depending on its solubility(1).

- **Sonication method**

This is the process that transforms the large Multilamellar vesicles into smaller Unilamellar vesicles. The MLVs get ultrasonic irradiation to obtain the SUVs(1). This approach uses a sonicator to sonicate lipids (MLVs). For the creation of liposome vesicles, two kinds of sonicators were employed: bath type sonicators and probe sonicators(2). Sonicators of the bath or probe variety are typically employed in an inert environment of nitrogen or argon. The principle involved in the sonication method is that high frequency, pulsed sound waves are employed to stir up the suspension of MLVs(3).

Probe type sonication method: In this technique, a titanium probe is used to transmit a lot of energy to the liposomal suspension. The major disadvantage is that thermo labile substances, such as proteins or DNA, become inactive or denatured. The discharge of titanium particles into the liposomal suspension may cause it to degrade.

Bath type sonicator: Bath type sonicators are mostly favored to address the problems with probe type sonicators. This technique involves placing a test tube holding an MLV dispersion in a bath-style sonicator. The MLV dispersion is sonicated for roughly 5 to 10 minutes at a temperature above the lipid's transition point. This results in the creation of a somewhat hazy, translucent solution, which is subsequently centrifuged to create SUV dispersion. MLVs and titanium particles combine to create a sediment during centrifugation. Later, the tube is taken out of the rotar, and the top clear liquid layer gets decanted with a Pasteur pipette, leaving behind the centre layer containing the MLVs and pellet. The pure SUV dispersion is in the upper layer(3).

- **French pressure cell**

The key aspect of the French pressure vesicle approach is that the protein does not appear to be greatly affected by the process of extruding MLV via a small opening as they are being sonicated throughout the operation. The process demands careful management of shaky techniques. The technique has compared to sonication, there are various benefits. The result the liposomes are bigger than the sonicated SUVs. The shortcomings are that it is challenging to use the procedure at high temperatures the working quantities are quite small and to attain (a maximum of around 50 ml)(7). The stainless-steel French pressure cell can resist pressures between 20,000 and 40,000 psi. The pressure chamber, pressure relief valve, piston, bottom seal, and valve closing are all found in the cell's body. Each rubber O-ring on the piston and the bottom seal is present. The cost of this approach is high, and cell cleansing is challenging. The piston is then pushed into the body using this technique, which entails adding liposomal suspension to the pressure chamber. The cell is then rotated 1800 degrees. After filled, the pressure chamber is sealed by pressing down on the bottom seal. The cell is then raised to its upright position and placed within a hydraulic press, which creates pressure. The valve is then gradually opened, releasing the substance (liposomes) is permitted to leave in a drop-by-drop 30-80 nm-sized ULVs or OLVs are produced(3).

- **Freeze-thawed method**

A variation of the conventional DRV method is the FTS method. A mostly SUV-based unilamellar dispersion is frozen by this process, which is then defrosted by letting it sit at ambient temperature for 15 minutes (in contrast to the DRV method, which rehydrates freeze-dried lipids with aqueous buffer) and subjected to a quick sonication cycle). As a result, the mechanism breaks down and rejects SUVs, allowing the solute to equilibrate between the interior and exterior of the liposomes, which also causes them to fuse and significantly enlarge. The entrapment volume may make up as much as 30% of the overall dispersion volume (10μl/mg phospholipids). Like DRV, empty liposomes are first prepared by sonication, and then, after freezing, they are briefly sonicated once more. The second sonication step significantly lowers the liposome membrane's permeability, maybe by speeding up the process by which packing flaws are corrected. The freeze-thaw process has been modified to include a dialysis phase against hypo-osmolar buffer in place of the second step sonication to prepare large vesicles with diameters between 10 and 50 m. In this instance, SUVS are first blended with a salt solution before being repeatedly frozen and thawed. The big vesicles created by freeze-thawing enlarge and break due to osmotic lysis during following dialysis, at which point they fuse with one another to produce a lot of giant vesicles[27].

- **Lipid film hydration method**

This is the most typical and popular technique for making MLV. For the preparation, a flask with a round bottom might be utilized. The process starts with the lipid solution being dried, followed by adding an aqueous buffer and vortexing the dispersion to hydrate the thin film. The hydration process is carried out at a temperature greater than the lipid's gel-liquid-crystalline transition temperature or greater than the transition temperature of the lipid mixture's greatest melting point. The molecules to be encapsulated are added to an organic solvent containing lipids or an aqueous buffer, depending on how soluble they are. The approach has drawbacks such poor interior capacity, ineffective encapsulation, and fluctuating size. By hydrating the lipids in the presence of organic solvents that are immiscible with one another, such as petroleum ether and diethyl ether, the low encapsulation efficiency can be overcome. Then it is sonicated to create an emulsion. By flowing nitrogen through the organic layer, MLVs are created(13).

- **Hand shaking method**

Lipid molecules must be added to an aqueous environment to generate liposomes. The lamellae swell and develop into myelin structures when the dry lipoid coating is hydrated. Myelin figures (thin lipid tubules) can only break and seal the exposed hydrophobic edges when mechanical agitation such as vortexing, shaking, swirling, or pipetting is applied. This results in the development of the liposome. The hand-shaken approach is a viable way to create large multilamellar liposomes(9).

- **Micro-emulsification method**

The micro emulsification technique produces tiny MLVs. The lipids are fed into the microfluidizer either as big MLVs or as a slurry of un-hydrated lipids in an organic medium using this approach. The fluid is pumped through a 5 mm aperture by a 10,000 psi microfluidizer. The fluid travels through the microchannels under this high pressure, which causes the two streams of fluid to collide at right angles and with greater velocities. Up until the production of the spherical vesicles, the fluid in this form is collected and circulated through the pump and interaction chamber. Vesicles shrink to between 0.1 and 0.2 m in diameter after a single pass(3).

- **Membrane extrusion method**

When putting MLVs through a membrane filter with a smaller hole size, they are shrunk in size using this technique. The membrane filter comes in two varieties. the nucleation track type and the winding path type. For sterile filtering, use the first. The fibers that make up this random path are crisscrossed. The average diameter of these fibres is determined by the density of these fibres in the matrix. Liposomes larger than the channel diameter strike the membrane when trying to cross such a membrane. The polycarbonate continuous sheet used in the nucleation track type is thin. Due to their precise diameter straight-sided pore holes that are bored from one side to the other, they will present less of a barrier to the passage of liposomes. Both LUVs and MLVs can be processed using this technique(4).

- **Dried reconstituted vesicles**

Initially, utilising a dispersion of freeze-dried empty SUVs, this process rehydrates it with an aqueous solution containing the substance to be trapped. This produces a finely divided dispersion of solid lipids. Instead of drying the lipids from an organic solution, the freeze-drying method is utilized to freeze and lyophilize a prepared SUVs dispersion. In contrast to random matrix structure, which can rehydrate, fuse, and reseal to produce vesicles with a high capture efficiency, this results in an orderly membrane structure. In order to ensure that the material for inclusion is present in the dried precursor lipid before the final addition of aqueous medium, the water-soluble components to be entrapped are added to the dispersion of empty SUVs and they are dried together. This approach typically yields unilamellar or oligolamellar liposomes with a diameter of 1.0 m or less. High water-soluble component entrapment and the use of moderate conditions for the preparation and loading of bioactives are two benefits suggested for the DRV approach. yet again due to the low integration rates with multilamellar vesicles, this approach is only appropriate for unilamellar vesicles like SUVs. Therefore, the liposomes that will be freeze-dried must be in the form of unilamellar vesicles[27].

3.2. Solvent dispersion method

In the solvent dispersion approach, lipids initially dissolve in an organic solution before being combined with an aqueous solution that contains the substances that will be included within the liposomes. The liposome's bilayer is made up of a phospholipid monolayer, which is formed when the lipids align themselves at the boundary between the organic and aqueous phases. The miscibility of the organic solvent and aqueous solution allows for the classification of solvent dispersion methods. These situations include those in which the organic solvent is miscible with the aqueous phase, the organic solvent is immiscible with the aqueous phase, and the organic solvent is both excessively present and immiscible with the aqueous phase[27].

- Ether injection method.
- Ethanol Injection method.
- Reverse Phase Evaporation techniques.
- Double emulsion method.

- **Ethanol injection**

Pushing MLV through a tiny hole at 20,000 pressure and 4 °C is part of the procedure. The approach has a few advantages over the sonication process. The process is quick, easy to repeat, and it handles unstable materials gently[20]. A huge amount of buffer is quickly infused with an ethanol lipid solution. The MLVs are created right away. The disadvantages of the method include the possibility of producing different biologically active liposomes containing the particularly lipophilic drug, as well as the population's heterogeneity (30-110 nm), the dilute nature of liposomes, the difficulty in eliminating ethanol because it forms an azeotrope with water, and the difficulty in removing all ethanol(1).

- **Ether injection**

Lipids are dissolved using this approach in diethyl ether or ether/methanol. The item to be enclosed is subsequently injected into an aqueous solution using this lipid combination. This is done at lower pressure or at a temperature of 55 to 650 C. By using vacuum, organic solvent evaporation is facilitated. Liposomes are eventually obtained(3).

- **Reverse Phase Evaporation techniques**

A rotating evaporator removes the solvent under reduced pressure while the lipid mixture is added to a flask with a flat bottom. The system is nitrogen-purged, and the organic phase—the phase in which the reverse phase vesicle would form—is then replenished with lipids. The two most used solvents are diethyl ether and isopropyl ether. The emulsion is created after the lipids have been re-dissolved, and the solvent is then removed by evaporating it into a semisolid gel while under reduced pressure. Then, unencapsulated material is taken out. Reverse phase evaporation vesicles (REV) are the liposomes that come from this process(8).

- **Double emulsion method**

The inner leaflet of the bilayer must be prepared first, followed by the outside half, to create liposomes using this procedure. The creation of water is one of this method's common characteristics. A modest amount of water-containing medium is added to an enormous volume of an immiscible organic lipid solution to create an oil emulsion. Mechanical agitation was then used to separate the aqueous phase into tiny water droplets. The phospholipid monolayer at the

contact helps to stabilize these droplets. The size of the droplet is determined by the ratio of lipid to aqueous phase volume and the amount of mechanical energy used to create the emulsion. Each droplet needs a complete monolayer of phospholipid covering its surface to prevent the possibility of coalescence with other droplets(2). Due to the collapse of some MVLs and drug leakage from the internal aqueous phase during solvent removal for the second emulsion, as well as the high temperature's promotion of lipid bilayer mobility and rearrangement, which leads to lipid fusion and the collapse of the aqueous chambers(17).

3.3. Detergent removal method

A variety of liposome and proteo-liposome formulations are prepared using the detergent depletion approach. By using a variety of methods, detergents can be removed from a mixed detergent-lipid micellar, resulting in the production of a very homogeneous liposome. With this method of production, practically any lipids below their phase transition temperature can be employed. Only a few detergents can be used for the detergent depletion procedure, and not all detergents are suitable for it. Alkyl (thio) glucoside, sodium cholate, and alkoxy polyethylene are the most widely used detergents. Multilamellar liposomes are combined with the concentrated detergent solution to create mixed micelles; the final detergent concentration should be significantly higher than the detergent's critical micelle concentration (CMC)(9).

4. Evaluation of liposomes

Liposomal formulation and processing are defined to ensure predictable *in vitro* and *in vivo* performance for a given application. Physical, chemical, and biological parameters make up the three basic categories into which the evaluation's characterisation parameters can be divided.

- Size, shape, surface properties, lamellarity, phase behaviour, and drug release profile are some of the aspects investigated by physical characterization.
- Chemical characterisation entails investigating the potency and purity of various lipophilic substances.
- Biological characterisation qualities are useful in establishing the formulation's suitability and safety for therapeutic application(4).

Table 2 Biological characterization

Characterization parameters	Analytical method
Sterility	Aerobic/anaerobic culture
Pyrogen city	Temperature (Rabbit) response
Animal toxicity	Monitoring survival of animals (rats)

Table 3 Chemical Characterization

Characterization parameters	Analytical method/Instrument
Phospholipid’s concentration	HPLC/Barrlet assay
Cholesterol concentration	HPLC / Cholesterol Oxide assay
Drug concentration	Assay method
Phospholipid’s peroxidation	UV observance
Phospholipid’s hydrolysis	HPLC/ TLC
Cholesterol auto-oxidation	HPLC/ TLC
Anti-oxidant degradation	HPLC/ TLC
PH	PH meter
Osmolality	Osmometer

Table 4 Physical Characterization

Characterization parameters	Analytical method/Instrument
Vesicle shape and surface morphology	Transmission electron microscopy, Freeze-fracture electron microscopy
Mean vesicle size and size distribution (submicron and micron range)	Dynamic light scattering, zetasizer, Photon correlation spectroscopy, laser light scattering, gel permeation and gel exclusion
Surface charge	Free-flow electrophoresis
Electrical surface potential and surface pH	Zeta potential measurements & pH sensitive probes
Lamellarity	Small angle X-ray scattering, 31PNMR, Freeze-fracture electron microscopy
Phase behaviour	Freeze-fracture electron microscopy, Differential scanning colorimetry
Percent of free drug/ percent capture	Mini column centrifugation, ion exchange chromatography, radiolabelling
Drug release	Diffuse cell / dialysis

4.1. Visual appearance

Liposome suspension can range from clear to milky depending on particle size and concentration. The appearance of a flat, grey tint implies the presence of a non-liposomal dispersion, which is most likely a scattered inverse hexagonal phase or distributed micro crystallites. Turbidity with a bluish tint indicates that the particles in the sample are homogenous. An optical microscope can detect liposomes larger than 0.3 m as well as contamination with larger particles(1).

4.2. Vesicle shape and lamellarity

The form of the vesicle can be assessed using electron microscopy techniques. Using freeze-fracture electron microscopy and p-31 nuclear magnetic resonance analysis, the lamellarity of vesicles, such as the number of bilayers present in liposomes, is determined(18).

4.3. Vesicle size and size distribution

There are many ways to identify size and size distribution. They are field flow fractionation, gel permeation and gel exclusion, light microscopy, fluorescent microscopy, electron microscopy (especially transmission electron microscopy), laser light scattering photon correlation spectroscopy, and other techniques. The ability to observe each individual liposome and collect precise data about the population profile of liposomes throughout the full spectrum of sizes makes electron microscopy the most accurate method of determining liposome size. Regrettably, it involves a lot of time and equipment that isn't usually accessible. The downside of the laser light scattering method, on the other hand, is that it is unable to gauge the average characteristic of the liposomes' bulk. All these methods require expensive tools. If only a general idea of the size range is required, gel exclusion chromatography processes are recommended because there are only two costs: buffers and gel material. A more recent innovation in microscopic techniques is atomic force microscopy, has been used to examine the morphology, size, and stability of liposomes. Most techniques employed in size, shape, and distribution study fall into one of four categories: microscopic, diffraction, scattering, or hydrodynamic techniques.

4.3.1. Microscopic Techniques

- **Optical Microscopy:** This technique for examining microscopic objects makes use of fluorescent, phase-contrast, and bright-field microscopes, and it is effective for determining the size of big vesicles.
- **Negative Stain TEM:** The two basic electron microscopic methods for assessing liposome form and size are negative stain TEM and scanning electron microscopy. The latter approach is less popular. Electron microscopy's negative stain is the representation of bright spots against a dark backdrop. In TEM analysis,

ammonium molybdate, phosphor tungstic acid (PTA), or uranyl acetate are utilised as negative stains. Uranyl acetate is cationic in nature, whereas PTA and ammonium molybdate are both anionic.

- **Cryo-Transmission Electron Microscopy Techniques (cryo-TEM):** The surface shape and size of vesicles have been clarified using cryo-TEM (cryo-Transmission Electron Microscopy) techniques.

4.3.2. Diffraction and Scattering Techniques

- **Laser Light Scattering**

The measurement of time-dependent intensity fluctuations in scattered laser light brought on by Brownian motion of particles in solution or suspension is known as photon correlation spectroscopy (PCS). Small particles disperse more fast than large particles, which also affects how quickly dispersed light's intensity changes. Thus, it is possible to determine the translational diffusion coefficient (D), and the mean hydrodynamic radius (Rh) of particles can then be determined using the Stoke-Einstein equation. With this technique, one may measure particles as small as 3 nm.

4.3.3. Hydrodynamic Techniques

To discriminate between SUVs and radial MLVs, exclusion chromatography on large pure gels was devised; however, huge vesicles of 1-3 m diameter often fail to enter the gel and are maintained on top of column. Although it wasn't tested, the use of agarose beads in a thin layer chromatography method has been established as a useful, rapid way for getting a general estimate of the size distribution of liposome production(4).

4.4. Determination of liposomal size

Typically, dynamic light scattering is used to measure it. For this approach, liposomes with a comparatively homogenous size distribution are reliable. Gel exclusion chromatography is an easy approach for identifying a hydrodynamic radius. Liposomes with a size range of 30-300 nm can be separated using sepharyl-S100. SUV and micelles can be separated using Sepharose -4B and -2B columns(13).

4.5. Drug entrapment studies

The volume was increased to 50 mL by adding 5 mL of 10% sodium lauryl sulphate (SLS) to a sample aliquot of liposomes (0.5 mL). The sample was heated for 30 minutes at 70°C in a water bath. Like this, a 50 mL volumetric flask was filled with distilled water after a 0.5 mL liposomal solution (drug-free) and 5 mL of 10% SLS were added to it. The blank was heated for 30 minutes at 70°C in a water bath. In a UV spectrophotometer, the test solution's absorbance was determined at 263 nm in comparison to the control solution. The ratio of the total amount of drug (in mg) to the amount of drug that is entrapped (in mg) can be expressed using the formula below(16).

Entrapment efficiency = Amount of drug entrapped /Total amount of drug X 100

4.6. Trapped volume

It is a significant factor that controls the shape of vesicles. The aqueous entrapped volume per unit amount of lipids is the trapped or internal volume. Between 0.5 and 30 microliters/micro mol may be used. A range of substances, including spectroscopically inert fluid, radioactive markers, and fluorescent markers, are used to calculate trapped/internal volume. Directly measuring the amount of water is the most accurate method for calculating internal volume by substituting a spectroscopically inert fluid (deuterium oxide) for the external medium (water), and then measuring the water signal using NMR. Trapped volume can also be determined experimentally by lipid dispersion in an aqueous medium containing a nonpermeable radioactive solute. Centrifugation is used to remove external radioactivity, and the residual activity per lipid is then calculated to indicate the percentage of solute trapped(4).

4.7. Liposome stability

Physically, chemically, and physiologically stable liposomes are ideal.

Physical stability reveals the medicinal drug to lipid ratio and the consistency of the size. Both the oxidative and hydrolytic degradation mechanisms have the potential to impact chemical stability. Most phospholipids in liposomes that transport unsaturated fatty acyl chains undergo phospholipid oxidation. In the absence of certain oxidants, these chains oxidise. Protection against light and oxygen, as well as low storage temperatures, helps reduce oxidation. Physical stability primarily refers to the consistency of the size and lipid-to-active-agent ratio. If adequately sterilised, the cationic liposomes can remain stable at 4 °C for a long time(1).

4.8. Drug release

Highly aligned *in vitro* dispersion cells can be used to investigate the medication component liberated from liposomes. The liposome-based definition can be aided by conducting *in vitro* trials to anticipate pharmacokinetics and bioavailability of medicine before using pricey and time-consuming *in vivo* investigations. To plan, a test that assessed intracellular drug release induced by liposome debasement in the presence of mouse liver lysosome lysate was used. As a gauge for the pharmacokinetic execution of liposomal details, the bioavailability of medication and another measurement that identified weakening prompted medication discharge in plasma and cushion were both utilised(18).

4.9. Surface charge

Since lipids that impart charge are typically used to create liposomes, it is important to understand how the charge on the vesicle surface is imparted. In general, zeta potential testing and free-flow electrophoresis are utilised to evaluate the charge. The surface charge on the vesicles can be inferred from the mobility of the liposomal dispersion in an appropriate buffer(1).

4.10. Phase response and transitional behaviour

Researchers are looking into the role that different phase transitions played in the stimulus-mediated fusion of liposomal components with the target cell or provoked drug release in liposomes and lipid bilayers. Producing and utilizing liposomes requires an understanding of phase transitions, the fluidity of phase transitions, and the fluidity of phospholipid membranes. because the permeability, fusion, aggregation, and protein binding of the liposomal membrane are all influenced by the phase behaviour of the membrane. Using freeze fracture electron microscopy, the phase transition has been assessed. By analysing data from a differential scanning colorimeter (DSC), they are more thoroughly confirmed(4).

5. Stabilization of liposome

Typically, liposomes can cause stability issues while being stored. In general, the following factors should be considered while formulating stable liposomal medicinal products:

- Using solvents and fresh, pure lipids throughout processing.
- Steer clear of high temperatures and a lot of shearing stress.
- Preservation of a low oxygen potential
- The use of metal chelators or antioxidants.
- Creating formulas with a pH of 7.
- Iso-protectant application during freeze-drying(13).

6. Application of liposome

Over the past 30 years, liposome research has undergone a significant expansion. Today, a variety of liposomes with different sizes, phospholipid compositions, cholesterol compositions, and surface morphologies that are suited for a variety of applications may be engineered. There are numerous methods that liposomes interact with cells to make liposomal components bind to target cells.

Using tomography, the liver and spleen can be the target of the liposome carrier, and normal tissue can be distinguished from tumour tissue. Liposomes have a lot of uses for transdermal drug delivery systems. The liposomal drug delivery system decreases side effects and boosts pharmaceutical effectiveness when used to target tumor cells. The liposome is guided to the site of action by coupling an amino acid fragment, such as an antibody, protein, or the pertinent fragments that target receptor cells. Some of the safest and perhaps most adaptable transfer vectors employed to date are liposomal DNA delivery vectors and their later development as LPDI -I and LPD-II. One recent use of liposomes is in DNA vaccination and increased gene therapy effectiveness(19).

6.1. Liposome in vaccination

When developing vaccines, cytoplasmic administration is essential for the induction of the cytotoxic T lymphocyte (CTL) responses that are essential in the fight against cancer and infectious illnesses. According to a publication, fusogenic liposomes can transfer antigens that have been encapsulated into the cytoplasm and trigger CTL reactions that are MHC class I restricted and antigen-specific. Furthermore, fusogenic liposomes work well as a mucosal vaccination delivery system. Macrophages and other reticuloendothelial system cells actively phagocytose liposomes. They therefore

function admirably as adjuvants for a variety of pure antigens. An exopolysaccharide or recombinant protein from bacteria are two examples that might be used to illustrate this idea. Even though these substances need expensive production and purification processes, a sufficient immune response can be produced when they are incorporated into liposomes in tiny doses(20).

6.2. Liposomes in sustained release drug delivery

Sustained release methods are required for drugs like cytosine arabinoside (Ara-C), which are rapidly removed *in vivo* but require plasma concentrations at therapeutic levels for a considerable amount of time for the optimum pharmacological effects. Now, sustained release liposome formulations with a prolonged circulation half-life and an ideal drug release rate *in vivo* are conceivable. As an effective prolonged release strategy, Ara-C encapsulated in LCL can be used to treat mouse L1210/C2 leukaemia, for instance. By gradually releasing medicines into the bloodstream, conventional liposomes that phagocytose into the cells of RES may function as a sustained release depot(21).

6.3. Liposomes used as target selective

The composition and structure of cell membranes play a significant role in how cells react. This can be used to target drugs by getting cells to respond to and take up the liposomes. To target a particular medicine, the membrane surface structure can be altered. This can be done by altering the membrane's charge or by incorporating proteins, antibodies, or immunoglobulins. It raises liposomes' affinity for cells. Creating liposomes that release a medicine only when exposed to a certain pH or temperature is one way to experiment with liposomes. It is possible to create liposomes that only interact with organisms. Site avoidance therapy, also known as liposome site avoidance, is used to reduce toxicity(14).

6.4. Liposomes in parasitic diseases and infections

Conventional liposomes are the best delivery systems for therapeutic molecules that target these macrophages because they are degraded by phagocytic cells in the body after intravenous administration. The most well-known examples of this "Trojan horse-like" process are several parasite illnesses that typically occur in the MPS cell. Leishmaniasis and numerous fungus infections are among them. The finest achievements in human therapy to date are presumably from using liposomes as for amphotericin B carriers in antifungal treatments. This medication is used to treat scattered fungal infections, which are frequently deadly and frequently co-occur with AIDS, chemotherapy, or the immune system. Unfortunately, the medicine itself is quite toxic, and its dosage is restricted because of its neurotoxicity and ionosphere. These toxicities frequently have something to do with how big the drug's molecule or complex is. Naturally, liposome encapsulation prevents drug build-up in these organs and significantly lowers toxicity(5).

6.5. Liposomes for brain targeting

The biocompatibility and biodegradability of liposomes make them ideal for use in drug delivery systems for the brain. Small-diameter (100 nm) and large-diameter liposomes freely diffuse over the BBB. However, tiny unilamellar vesicles (SUVs) attached to brain drug delivery systems may cross the BBB via receptor- or absorptive-mediated transcytosis. Cationic liposomes are endocytosed by absorptive mechanisms into cells, but it is unknown whether they are also transcytoses by absorptive mechanisms across the BBB. Mannose-coated liposomes can penetrate the brain and help the BBB carry loaded medicines. The neuropeptides leuenkephaline and mefenkephalin kyoforphin often do not cross the blood-brain barrier when given systemically. The BBB is frequently penetrated by the antidepressant amitriptyline because to the flexibility of this method(1).

6.6. Liposomes in anticancer drug formulations

The first line of treatment for cancer is chemotherapy. However, most of them have limitations because of their obvious toxicity, poor tissue selectivity, restricted therapeutic indices, and high likelihood of developing drug resistance. These elements may cause cancer treatments to fail horribly. It has been demonstrated that the creation of nanoscale liposomal formulations aids in the drug's targeted delivery to cancer cells. As a result, the off-target toxicity brought on by the EPR effect is avoided. Some liposomal-based DDS with strong anticancer efficacy were authorised by the FDA. DOX is an anthracycline antibiotic with strong anticancer properties that is frequently used to treat solid and hematologic neoplasms (such lymphoma and breast cancer). However, due to significant cardiotoxicity and cytotoxicity, its clinical uses are restricted. Due to the production of free radicals and lipid peroxidation, cardiotoxicity is typically cumulative and primarily causes irreparable damage to cardiomyopathy and congestive heart failure(22).

6.7. Liposome in nucleic acid therapy

The efficient delivery of nucleic acid into cells *in vitro* and *in vivo* is essential for recombinant DNA technologies, gene function research, and gene therapy. Non-viral vectors will be developed to deliver the gene only to malignant cells. By

connecting a nutrients ligand to the vector (liposome), the vector will benefit from the increased nutritional requirements of rapidly developing cells. A special pH-sensitive surfactant and a passively charged lipid will also be present in the vector to enhance nucleic acid binding. Surfactants can boost the amount of nucleic acid that leaves the endosome, which in turn improves transfection efficiency(23).

6.8. Liposomes in cosmetics

One new technology in recent years in the pharmaceutical and cosmetic industries is the liposome. The existence of biological carriers, such as liposomes packed with active substances, is extremely useful for treating illness and caring for the skin. Freeze fracture electro micrography's findings indicated the presence of liposomes. Measuring the liposome's particle size and microscopic structure, testing the rate at which they encapsulated the water-soluble material, and discussing their use in cosmetics led to the predetermined conclusion that they had good stability. The first Nano-Pharmaceuticals on the market are liposomal formulations; the first one is Doxil® PEGylated liposomal formulation for doxorubicin. These liposomes, which have a diameter of 200 nm and a hydrophilic (PEG) surface, go by the name "Stealth" liposomes. The increased permeation and retention (EPR) mechanism was discovered to be responsible for this long circulating liposome's ability to target cancer tissue. Thus, the cardio-toxicity of the medication was significantly decreased by the liposomal formulation of doxorubicin(5).

6.9. Liposomes in gene therapy

In addition to being employed for medication and gene delivery, liposomes are frequently used in the analytical sciences. A few systemic disorders are brought on by a deficiency in enzymes or other components that results from missing or broken genes. By introducing the appropriate exogenous DNA or genes into cells, various attempts have been undertaken to restore gene expression in recent years(1).

6.10. Nanoliposome therapeutics in red blood cells

A brand-new method of medication delivery that involves electroporating nanoliposome therapies into human red blood cells (RBCs) to improve *in vivo* drug circulation. A study, which involved a thorough analysis of the delivery method, revealed that the precise type and concentration of liposomes, the number of electroporation pulses used, and other factors are crucial considerations for effectively loading nano systems in RBCs. In an effort to extend the life of the cells after reinjection, they also examined the impact of a cell preservation solution on the survival of electroporated red blood cells(5).

6.11. Liposome as anti-infective agents

The liver and spleen are home to intracellular pathogens such protozoa, bacteria, and fungi; therefore, to eradicate these pathogens, the therapeutic agent may be targeted to these organs employing a liposome as a transport system. The related therapeutic drug uses liposomes to target diseases including leishmaniasis, candidiasis, aspergillosis, histoplasmosis, erythrocytosis, giardiasis, malaria, and tuberculosis(8).

6.12. Liposome in eye disorders

In the past, liposomes have been employed to treat problems of both the anterior and posterior region. Among the eye illnesses are dry eyes, keratitis, corneal transplant rejection, uveitis, endophthalmitis, and proliferative vitreous retinopathy. Retinal diseases are the leading cause of blindness in affluent countries. Both a genetic transfection vector and a monoclonal antibody-targeted vehicle are formed of liposomes. The most modern methods of therapy, such as targeted laser application to heat-induced liposomal medication and dye release, are used to treat selective cancer and neo-vascular artery blockage, angiography, retinal and choroidal blood vessel stasis, and other conditions. To present, two patent medicines have been approved for liposomal medicinal compositions, and several other items are undergoing clinical studies. The benefit of the liposome will be employed in the treatment, diagnostic, and research aspects of ophthalmology in the future, with "verteporfin" being one of the liposomal medications that is now approved for use in the eye(19).

6.13. Liposomes for respiratory drug delivery system

In many different forms of respiratory illnesses, liposomes are frequently employed. Liposomal aerosol is superior than conventional aerosol in several ways:

Improved stability in a large aqueous core, prolonged release, prevention of local irritability, and reduced toxicity. Currently available injectable liposomal products include Ambisome, a few fungi, and mycoses. The following factors

affect how effectively a liposomal structure delivers drugs to the lungs: Drug/lipid relationship, lipid composition, size, loading, and delivery method(16).

7. Challenges of liposomes

Drug formulations based on liposomes have not been widely available up to this point. Stability concerns, batch to batch reproducibility, sterilisation technique, low drug entrapment, particle size control, manufacturing of high batch sizes, and short circulation half-life of vesicles have been some of the issues restricting liposome manufacture and development. Numerous clinical trials and new approvals have resulted from the resolution of some of these problems, such as the short half-life. Below, we go into further detail about a few of the outstanding issues(21).

7.1. Sterilization

Due to liposomes' sensitivity to extreme heat and some types of radiation, sterilising them is a challenging problem. Chemical sterilisation is not an option either because it can compromise the liposomes' stability. There is just one way of filtering through a 0.22 m membrane filter for the creation of sterile liposomes. If the liposomes are larger than 0.2 m in diameter, this approach is not appropriate since it does not get rid of viruses. Before beginning production, another option is to filter the initial solutions through 0.45-m regenerated cellulose filters and glass fibre filters. After that, the entire production process must be carried out in an aseptic environment(14).

7.2. Encapsulation efficiency

Since lipids in high doses may be toxic and result in non-linear (saturable) pharmacokinetics of liposomal drug formulation, liposome formulation of a drug could only be developed if the encapsulation efficiency is such that therapeutic doses could be delivered in a reasonable amount of lipid. There have been several recent developments that offer hydrophilic medicines significant encapsulation efficiencies. To improve encapsulation efficiency, for example, mild acidic or basic amphipathic medicines may be actively loaded into empty liposomes. However, due to the hydrophobic drug's low affinity for the lipid bilayers and encapsulation effectiveness of paclitaxel, which is just 3 moles%, active loading is not appropriate(21).

7.3. Short shelf life and stability

A pharmaceutical product needs to stay stable in some capacity for at least one and a half to two years to be commercially successful. If the liposomes are in suspension, it is quite challenging to accomplish this. Freeze drying can be utilised following manufacture to increase the liposomes' self-life. Two main factors significantly influence the Specifically, chemical, and physical degradation affect liposome stability. They chemically deteriorate through oxidation and hydrolysis. to lessen only brand-new, fresh, and high-quality reagents for oxidation and hydrolysis Quality is employed, low-temperature techniques are avoided, and use Deoxygenating aqueous solutions and storing liposomes in an inert environment and conduct all manufacturing in an oxygen-free environment. Finally, you might include an antioxidant such -tocopherol. The variation in lipid packing density in the bilayer structure is frequently blamed for physical deterioration. By keeping the liposomes at a temperature around the phase transition temperature until the lipid arrangement equalises, this can be corrected. Liposomes frequently fuse together, which is what causes the instability of this structure. Cholesterol is added to the lipid mixture to increase the transition temperature, which reduces instability(14).

7.4. Nucleic acid therapeutics

It is believed that nucleic acid treatments have the potential to alter the application of personalised medicine. The potential to treat diseases exists with these treatments. *In vitro* results for nucleic acid therapies are encouraging, but there are still numerous obstacles to be cleared. The creation of a successful distribution system is a major task. Both viral and non-viral delivery methods are being explored, with non-viral ones receiving the most attention. Despite the significant improvements made in nucleic acid delivery, particularly with liposomes, there are still many issues that need to be resolved. These include:

- The stark differences between *in vivo* testing of delivery systems and the mostly employed *in vitro* models in research.
- Even though PEGylated Liposomes were used to help with the short circulation times, studies revealed that even this type of Liposome still had rapid clearance after several injections.
- The therapeutic use of nucleic acids still faces the problem of serum instability(6).

8. Technology transfer

Prior to their technology transfer and market release, pharmaceutical nano formulations and health care products must undergo a mandated quality assessment. Even though more and more liposome systems can demonstrate their favourable effects in preclinical studies, only liposome nano formulations that are successful in clinical studies will be used in patients. Many distinct liposome formulations have successfully entered the clinic over the past few decades, while others are still undergoing various stages of clinical research. Pharmaceutical products' performance, stability, and lack of contamination are all closely related to their quality, which is confirmed by their effectiveness in achieving the therapeutic benefits stated on the labels of the items. Before liposome nano formulations are recognised (and marketed) as biomedical products, a set of physicochemical and biopharmaceutical characterization methods must be used because many of them make use of novel drugs and have complex interactions between the different molecular components. The high production costs and onerous standards for evaluating their advantages (or hazards) may act as a barrier to an effective technology transfer. The precise specifications of a liposome nano formulation rely on the pharmaceutical intervention type, the patient group that it is intended for, and the appropriate administration route. In this regard, the regulatory bodies specify the various criteria necessary for the quality, safety, and efficacy of the liposome-based products(24).

9. The regulatory landscape

There are two parts to this section. In the first, we discuss the regulatory experience with the market approval of the innovator's liposome drug products, and in the second, we discuss the market approval of generic equivalents.

9.1. Liposomes in new drug applications

9.1.1. Europe (EMA)

According to the EMA Volume 2B Notice to Applicants Medicinal Products for Human Use; EMA ICH Topic M 4, the Common Technical Document (CTD) with a layout as described in the ICH topic 4 document is used for submission of a dossier requesting marketing authorization of new liposomal drugs via the "central procedure." In other words, the whole dossier as outlined in the ICH topic 4 document is required to be provided, consisting of 5 modules: regional administrative information (module 1), summaries (module 2), quality aspects (module 3), nonclinical research findings (module 4), and clinical reports (module 5). To educate healthcare professionals about "the why and how to use it," EMA provides a SmPC (Summary of Product Characteristics) after receiving a marketing authorization. This 'live document' is continually updated, for example, by the addition of new indications. The EMA website has SmPCs for drugs like Caelyx, Myocet, Daunoxome, Mepact, and Visodyne.

9.1.2. United states (USFDA)

Even though the medicine (without the liposome) has been in use for a long time, the section 505(b)(1) protocol-a full NDA-must be followed for new drug-liposome combinations. As a result, two controlled clinical trials must be conducted to support approval. To demonstrate the anticipated successful therapy outcome, research must be conducted. Instead of using the entire NDA, one could think about using 505(b)(2) in this situation. Hybrid/mixed market application procedures in Europe and 505(b)(1) in the US. These regulatory approval processes serve as bridges between novel and generic application of drugs. Liposome product development is much more cost-effective than creating a new chemical entity (NCE) since they enable referring to existing dossiers of authorised active pharmaceutical ingredients (API) administered in different dosage forms, thereby lowering the number of clinical studies required. For instance, Doxil was approved through the 505(b)(2) method and referred to the doxorubicin dossier, while Depodur referred to the morphine sulphate dossier.

9.2. Generic versions or other liposomal drug products

9.2.1. Europe (EMA)

Liposome generic versions have not yet received EMA approval. The EMA declined a request for a generic version of Doxil/Caelyx (Lipodox). The assessment report states that "there are significant unresolved major nonclinical and clinical objections that currently preclude a recommendation for marketing authorization." The goal of TLC's (Taiwan Liposome Company) bioequivalence tests in Europe with its Doxil generic (DoxisomeTM) is to obtain EU market approval by the end of 2015, following that, it will keep working towards Doxisome's bioequivalence and US market approval. The Taiwan Liposome Company (TLC) produced the AmbilTM generic version of the brand-name drug Ambisome, which has been given local approval for the management of invasive infections. The EMA Ambil generic

application is anticipated to be submitted by TLC by the end of 2014, with the marketing authorization process for EU nations taking a further 6 to 9 months.

9.2.2. United states (USFDA)

The FDA released a "Draught guidance on doxorubicin hydrochloride liposomes" in 2010 that included non-binding suggestions for the manufacturers of generic versions of reference list doxorubicin-containing liposomes (Doxil). The guidelines are intended for generic products where the reference and test PEGylated liposome products:

- They have the same drug product composition
- Produced using an active liposome loading process with an ammonium sulphate gradient
- They have equivalent liposome characteristics, such as liposome composition, state of drug encapsulation, internal environment of the liposome, liposome size distribution, number of lamellae, and grafted PEG at the liposome surface.

In 2013, the FDA authorised the sale of LipodoxTM, a generic form of DoxilTM, under the Abbreviated New Drug Application (ANDA) process. Due to a lack of DoxilTM, the FDA first granted a temporary permit for the use of LipodoxTM. Also offered in India is LipodoxTM.

9.2.3. Rest of the world

Amphotericin liposomes other than Ambisome have been authorised for sale outside of the EU and the US. The same holds true for Doxil and doxorubicin liposomes, mutatis mutandis. Sometimes it's unclear if these businesses are trying to create a generic version of Ambisome or Doxil (sameness principle) or if they've come up with a brand-new formulation. As previously indicated, there are a few (generic) amphotericin-liposomes (reference product: Ambisome™?) available on the market under brands like Lambin Liposome™—Sun Pharma India and Fosome™—Cipla, India (personal communications). TLC markets AmbilTM in Taiwan.

FungisomeTM—Lifecare is sold in India. The requirement to sonicate this amphotericin-lipid formulation before to delivery is a noteworthy characteristic in the realm of parenteral liposome products. Numerous clinical investigations have been carried out and documented. AnfogenTM is an amphotericin liposome that is made using a different manufacturing process, yet it has a comparable lipid content as Ambisome. The Argentine government has approved Anfogen, which is marketed by Genpharma S.A., Argentina. compared the toxicological, antifungal, and physicochemical characteristics in animals and *in vitro*. Their research demonstrates the differences in the physicochemical characteristics of Ambisome and Anfogen. Anfogen is not a generic equivalent of Ambisome, for this reason. The generic version of Ambisome is being examined by the FDA, but no new information has been released yet. Gaspani and Milani suggest that the WHO take a proactive role in developing protocols to guarantee high-quality, generic versions of amphotericin liposomes to promote and expand access to reasonably priced amphotericin liposome products globally. Visceral leishmaniasis is a dangerous, sometimes fatal illness that affects regions of Asia and Africa. The formulation now on the market is quite effective against this disease. Perhaps an evaluation of generic alternatives utilising the World Health Organization's (WHO) prequalification system approach could be taken into consideration. "In close cooperation with national regulatory agencies and partner organisations, the Prequalification Programme aims to make quality priority medicines available for the benefit of those in need," reads the mission statement of this WHO programme. This is accomplished through strengthening national capacity for sustainable manufacturing and monitoring of high-quality pharmaceuticals, as well as through its review and inspection activities(25).

10. Liposomal formulations that are approved by the EMA and USFDA

10.1. Ambisome

Amphotericin is used in this formulation and the lipids used are Hydrogenated soy bean phosphotidylcholine (HSPC), Distearoylphosphatidylglycerol (DSPG), Cholesterol. The freeze-dried method was used for the formulation. The size ranges below 100nm. It is introduced by EMA in 1990 and by FDA in 1997. The patent was expired in 2016. The license holder is Astellas and the manufacturer is Gilead sciences.

10.2. DaunoXome

The drug used for this formulation is Daunorubicin, with the lipids used are that distearoylphosphatidylcholine (DSPC) and cholesterol. Aqueous dispersion method is used as the formulation method. Size ranges between 40 to 80nm. It was introduced in 1997 by EMA and USFDA. The license holder is Galen and Gilead sciences.

10.3. DepoCyt

Cytarabine and the dioleylphosphatidylcholine (DOPC), dipalmitoylphosphatidylglycerol (DPPG) is used as the API and the lipids. The method followed is Aqueous dispersion and its size range is 20µm. In 1999 it is introduced in US and the license holder Sigma-Tau pharmaceuticals and the manufactured by Pacira.

10.4. DepoDur

Morphine is used as the API and the lipids used are DOPC and DPPG. Aqueous dispersion method is carried out for the formulation. Size ranges between 17 to 23 µm. It was introduced in 2004 in US. In UK the license holder is Flynn Pharma Ltd and manufacturer is Almac Pharma Services Ltd, Ireland.

10.5. Doxil/Caelyx

The API used is Doxorubicin with the lipids like HSPC, cholesterol and distearoylphosphatidylcholine polyethyleneglycol (DSPE-PEG). Aqueous dispersion method is carried out for the formulation. Size range is 100nm. This was introduced in US by 1995 and in 1996 by EMA. Jansen-Cilag is the manufacturer and the license holder.

10.6. Exparel

Bupivacaine is used as an API. DOPC, DPPG and tricaprylin are used as lipids in the aqueous dispersion method. 24 to 31 µm is the size range which introduced in the year 2011 by USFDA. Pacira is the license holder and the manufacturer.

10.7. Lipodox

The API used is Doxorubicin with the lipids like HSPC, cholesterol and distearoylphosphatidylcholine polyethyleneglycol (DSPE-PEG). Aqueous dispersion method is carried out for the formulation. Size range is 100nm. Introduced as the generic version of Doxil/Caelyx in 2013 by USFDA which is manufactured by the Sun Pharma.

10.8. Marqibo

The drug vincristine is used with the lipids like cholesterol and sphingomyelin (SPH) then leads to the formulation of liposomes by the freeze-dried method. Introduced by USFDA in 2012. The licensed holder and manufactured by Talon Therapeutics.

10.9. Mepact

Mifamurtide is used as the API and the lipids that are used are DOPC, dioleylphospatidylserine (DOPS). The freeze-dried method is used for the formulation which introduced in 2009 by EU. Takeda is the license holder and the manufacturer.

10.10. Myocet

Doxorubicin is used as the active ingredient with lipids like egg phosphatidylcholine (EPC) and cholesterol by freeze-dried method. The size range between 80-90nm which introduced in 2000 by the EMA. TEVA is the license holder and the manufacturer.

10.11. Visudyne

The API is the Verteporfin with the lipids like sEPC and DMPC by the freeze-dried method of formulation. It has the size range between 18-104nm. It is introduced in 2000 by US and EU. Valeant holds the license and the manufacturer(25).

12. Future prospective

Although nanotechnology has advanced to new heights, pharmacological and kinetic investigations on tumours are difficult due to heterogenicity and complicated tumour anatomy. Furthermore, it is challenging to create tumour-imitating 3D spheroid models, which are typically implicated in cancer metastasis and the development of drug resistance inside the body. This makes *in vitro* studies on cancer treatment extremely challenging. Accuracy is required in both the diagnostic process and the treatment strategy. Theranostic approaches are increasingly in demand because they provide real-time imaging along with therapy, allowing for more individualised phytomedicine monitoring and response data, but their complexity further hinders regulatory approval. The regulatory elements of phytochemical therapy in combination with synthetic chemotherapeutic drugs may also continue to receive attention because they will significantly improve the formulation's safety, efficacy, and stability(26).

13. Conclusion

This review concluded that liposomes are a promising carrier for improving the targeted delivery of a range of drugs. An extensive spectrum of medicinal applications has made use of liposomes. Liposomes are acceptable and superior carriers which is one of the most well-known and practical carriers for regulated and precise drug delivery systems is the liposome. To encapsulate amphiphilic medications and shield them from deterioration, liposomes are acceptable and superior carriers. These systems can be given via topical, parenteral, and oral modes of administration. Currently, a range of medications are transported through liposomes. Despite a few drawbacks, liposomes are effective drug delivery systems for a variety of substances. The utilization of liposomes to carry genes and medications is a promising strategy that will undoubtedly advance in the future.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Reference

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