WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 9, 2926-2950. Review Article ISSN 2277– 7105

LIPOSOME AS DRUG DELIVERY SYSTEM: AN OVERVIEW AND THERAPEUTIC APPLICATION

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Article Received on
21 April 2023,

Revised on 11 May 2023,
Accepted on 01 June 2023

DOI: 10.20959/wjpr20239-28508

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ABSTRACT

Backgroungd: Over the past 30 years, the liposome, a closed phospholipid bilayer vesicular structure, has drawn a lot of attention as a medicinal carrier with enormous promise. To improve treatments, powerful drugs have been formulated using liposome drug delivery methods. Aiming to decrease toxicity and increase accumulation at the target region, liposome formulations have recently been developed. **Objective:** Liposomes are appealing drug delivery vehicles due to their capacity to encapsulate both hydrophilic and hydrophobic medicines as well as their biocompatibility and biodegradability. The majority of therapeutic uses for liposomal drug delivery target tissue, whether or not target identification molecules are expressed on the semipermeable bilayer. The physical, chemical and biological characteristics of the liposomes are described. For a variety of pharmaceuticals, liposomes are being created for drug delivery, and they have been crucial in the development of powerful medications that have improved therapy. Initially employed to research cell biological behavior, they later evolved into a drug delivery system for focusing on a particular region of action, such as tumor targeting, gene and antisense therapy, genetic immunization, and cosmetic, among other things. **Conclusion:** There is a bright future for liposomes in the pharmaceutical industry. This review aims to investigate the liposome drug delivery system, including its production process, characterization, and various therapeutic uses.

KEYWORDS: Liposomes, Drug delivery system, Phospholipid, Liposome preparation, characterization.

BACKGROUND

The Greek word "liposomes" means "fat" and "somes" means "body".[1] Dr. Alce D. Bangham, a British hematologist, first defined liposomes as synthetic in England in 1961. His findings were published in 1964.[2] Liposomes are just spheres or "bags" with a colloid structure and a lipid-based membrane that contain water divisions. The aqueous compartment of liposomes, which are concentric bilayer vesicles by structure, is encased by the bilayer membrane, which is made up of both natural and synthesized phospholipid bilayer.[3,4] Such vesicles can contain lipid-soluble medications within membranes and liquid drugs within their watery spaces, including peptides and proteins, hormones, enzymes, antibiotics, antifungal, and anticancer compounds.[5] Liposomes are typically described as spherical vesicles that can vary in size from 30 nm to several micrometers. Liposomes may contain a wide range of molecules, from those with low molecular weights like glucose to those with high molecules like peptides and proteins.[6]

It has been demonstrated that when phospholipids are hydrated into aqueous systems, closed forms spontaneously form. Depending on the type of drug being transported, such vesicles with one or more phospholipid bilayer membranes can either transport aqueous or lipid pharmaceuticals. Lipids are amphipathic (both hydrophobic and hydrophilic) in aqueous conditions, and this causes the entropically focused packing of their hydrophobic portions into spherical lipid membranes to be influenced by the thermodynamic phase properties and self-assembling features of lipids. Lamellae are the name for such strata.[7] As circular vesicles have particle sizes that range between 30 nm to several μm in diameter, liposomes are typically identifiable. The polar head groups are positioned in the path of exterior and interior aqueous phases and are made up of one or more lipid bilayer membranes surrounding aqueous units.[8] However, polar lipids can self-assemble into a variety of colloidal particles in addition to the usual bilayer forms that depend on the molecule shape, temperature, and environmental and preparation circumstances.[9]

In the cosmetics and pharmaceutical industries, liposomes are widely employed as molecule carriers.[10] The utilization of liposome encapsulation to develop delivery methods that can entrap unstable substances (such as antimicrobials, antioxidants, flavors, and bioactive components) and safeguard their activity has also been thoroughly researched by the food and

farming industries. Both hydrophobic and hydrophilic molecules can be captured by liposomes, which can also prevent the combination's breakdown and release the trapped substances at specific destinations.[11] Due to their capacity to capture both hydrophilic and lipophilic medicines as well as their biocompatibility, biodegradability, low toxicity, and other properties.[12] They can make easier administration of tumor tissue medication at specific sites.[13] Both as an experimental system and commercially, liposomes have become more popular as a means of medication administration. A lot of research has been done on liposomes to reduce drug toxicity and/or target particular cells.[14,15,16]

Because of their structural flexibility, biocompatibility, degradability, non-toxic nature, and lack of immunogenicity, liposomes are regarded as effective drug delivery platforms.[17] Since phospholipids in solution have an amphiphilic nature similar to that of natural cell membranes, liposomes can effectively interact with mammalian cell membranes to promote cellular absorption. The large pharmacological payload carrying capacity, self-assembling ability, and a variety of physicochemical and biophysical features that may be altered to influence their biological characteristics are all additional benefits of liposomes.[18] By stabilizing therapeutic compounds, removing barriers to cellular and tissue absorption, and enhancing biodistribution of drugs to target areas in vivo, liposomes have enhanced therapeutics for a variety of biomedical uses.[19] The medication is shielded from physiologically occurring events such as enzymatic degradation, chemical and immunologic inactivation, and rapid plasma clearance, which helps to improve and extend the activity of the drug when it is put into a liposome.[20]

Advantages

Reduce exposure by passively targeting cancer and selected tissue with toxic drugs. Enhanced therapeutic index and effectiveness. Improves formulation stability as a result of encapsulation. decrease in the encapsulating agent's toxicity. Vesicles are entirely biodegradable, completely biocompatible, and neither immunogenic nor poisonous.[21] Provides (Liposomal Doxorubicin) selective passively targeting malignant tissues.[22] Improved therapeutic index and efficacy. additional stability through encapsulation. Decrease in the encapsulated drugs' toxicities. Effect of site avoidance. Better pharmacokinetic results (reduced elimination, increased circulation lifetimes). Flexibility to combine with ligands that target a specific location to accomplish active targeting.[1]

Main text

Design of liposome

There are various ways to make lipid membranes, and each one affects different aspects of them, such as their size, lamellarity, and encapsulation effectiveness (EE). While some methods are simple, especially when used in the lab, others are more beneficial when scaled up yet call for specialized equipment. The techniques might be classified as standard or unique, as we shall see. We'll talk about how to sterilize the liposome preparations and how to load drugs into the liposomes.[6]

Molecular component of liposomal drug delivery system

Natural or synthetic lipids, polysaccharides, sterols, or surfactants can be used to create liposome compositions. Research on liposome formulation has led to the development of unique properties such as improved drug encapsulation, stimulus responsiveness, tissue targeting, prolonged blood circulation, reduced drug toxicity in non-target tissues, and diagnostic capabilities, to name just a few. When compared to giving out free medication, these qualities increase treatment efficacy in a variety of clinical applications. All the components of the liposome are shown in Fig.1.[7]

Natural lipids

Lipids have a wide range of structural variations, but they always contain a hydrophilic term in order and a hydrophobic, hydrocarbon tail in common. The headgroup may be zwitterionic, positively charged, or negatively charged (having both a negative and positive charge, resulting in net neutrality). It is also possible to chemically alter the headgroup to enable conjugation with other molecules. For instance, the amine group is frequently used to conjugate phosphatidylethanolamine (PE) to polyethylene glycol. Since charged liposomes repel one another electrostatically, the headgroup's charge confers stability. The length of the acyl chain, symmetry, and saturation of the hydrophobic tails can all vary. Typically, a phosphate, glycerol, or sphingosine group serves as the lipid's backbone.

Phospholipids

The primary fundamental molecular building block of biological membranes, including cell membranes, are liposomes. Three different phospholipid kinds exist (along with their hydrolysis product). These are Phosphoglycerides, Sphingolipids, and lipoprotein.

The most prevalent phospholipid is phosphatidylcholine (pc), which is not soluble in water or aqueous media. Phosphatidylcholine molecules are aligned in a bilayer sheet to minimize the unfavorable interaction between the bulk aqueous phase and the long hydrocarbon fatty chain.

There are 3 main components to each phospholipid molecule. Two tails, one head Choline, phosphate, and hydrophilic glycerol make up the three molecules that make up the head. Each tail has a lengthy chain that has a hydrophobic character.[19]

The phospholipid with glycerol that makes up the majority of the weight of the lipid in biological membranes and is most frequently employed in liposomes is generated from phosphatidic acid.[23]

Examples of phospholipids that are commonly used:
Phosphatidylinositol (PS)
Phosphatidylethanolamine (PE): Cephalin
Phosphatidylchloine (PC): Lecithin
Phosphatidyl glycerol (PG)
Phosphatidylserine (PS)

Sphingolipids

Sphingolipids are an additional natural lipid class that predominately exists in the membranes of mammalian cells. Sphingolipids, which have an 18-carbon amino-alcohol sphingosine backbone and are essential for the formation of cell membranes as well as regulatory signaling molecules, are lipids.[24] Sphingolipids, such as sphingomyelin (SM), have been added to liposomal formulations to enhance blood circulation, stability in vivo, and therapeutic effectiveness. Sphingolipids called ceramides are made up of sphingosine and a single fatty acid. Ceramides are added to liposomal formulations to improve membrane fluidity and cutaneous cell fusion potential.[25]

Sterols

A type of natural lipid compound called sterols is found in almost all living things. Phytosterols, Zoosterols, and Mycosterols are the three subtypes of sterols, and they are correspondingly found in plants, animals, and microbes.[26] In mammalian cell membranes, cholesterol plays a crucial role as an endogenous amphiphilic zoosterol. Cholesterol is

predominantly restricted to lipid rafts within the cell membrane and plays a significant role in mechanisms that control membrane integrity and lipid raft activity.[27]

The membrane is stabilized by cholesterol, which also aids in the creation of bilayers. Although cholesterol does not naturally form a bilayer structure, it can be added in very high concentrations—up to a 1:1 or even 2:1 molar ratio of cholesterol to phosphatidylcholine—to the phospholipid membrane. Cholesterol functions as a fluidity buffer, improving the membrane's stability. The freedom of movement of the carbon molecules in the acyl chain is altered after intercalation with the phospholipid molecule.[28] Normal electrical and hydrogen bonding interactions are eliminated and the gap between the choline head group is increased by cholesterol inclusion.[29]

Polysaccharides

Long-chain polymeric carbohydrates known as polysaccharides are made up of monosaccharides joined together by glycosidic bonds. Polysaccharides are found in cell membranes to help with processes of cell and tissue recognition, as well as specific transport mechanisms, and they play a significant part in cellular communication.[30] Liposomes may be directed to cell receptors or their circulation may be increased by coating the lipid membrane with oligo- or polysaccharides.[31,32] Additional characteristics of polysaccharides, such as biocompatibility and antiviral, antibacterial, and anti-tumor activities, make them suitable for application in drug delivery systems.

Surfactants

Surfactants are categorized as molecules that lessen the liquid's surface tension when they are included. In liposome formulations, surfactants also known as edge activators—are helpful additions. Edge activators, which are typically single acyl-chain surfactants, work to weaken the lipid bilayer of liposomal nanoparticles, which increases vessel deformability.[33]

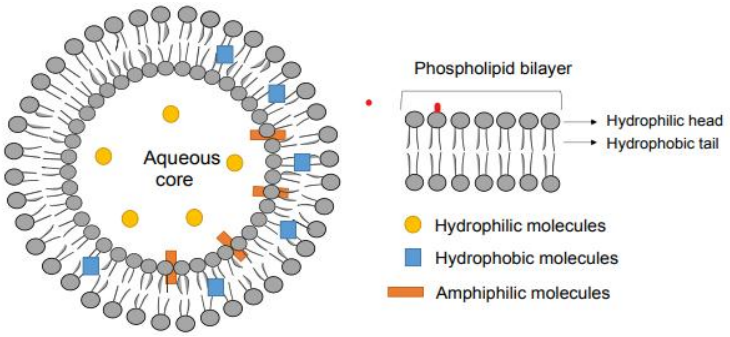

Fig. 1: Structure of liposome.

Mechanism of liposomes formation

The amphiphilic molecule, which is generated by the phospholipids, is the fundamental component of liposomes. When these phospholipids are in an aqueous media, they organize so that the hydrophilic polar head group extends outward from the aqueous region, resulting in aggregated complexes. The two fatty acid chains with 0–6 double bonds and 10–24 carbon atoms each make up the majority of the hydrophilic portion.[31] The hydrophilic fraction, which primarily consists of phosphoric acid, is in touch with the aqueous region and conflicts with the hydrophobic non-polar portion.[34] When phospholipids are hydrated in water, one or more bilayers will consequently develop with the addition of energy such as sonication, shaking, heating, homogenization, etc., each separated by a water molecule. This causes lipid-lipid and lipid-water molecules to interact hydrophobically and hydrophilically, forming bilayer vesicles and bringing the aqueous phase into thermodynamic equilibrium.[33]

Classification of liposomes

From extremely small (0.025 m) to big (2.5 m) vesicles, liposome size can vary. Table 1 list the benefits and drawbacks of liposomes.[35] Benefits of liposomes-the drawbacks of liposomes Drug effectiveness and therapeutic index increased thanks to liposomes (actinomycin-D) minimal solubility Liposomes' encapsulation improved stability. a short half-life For both systemic and non-systemic treatments, liposomes are non-toxic, adaptable, biocompatible, totally biodegradable, and non-immunogenic. Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction. Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol) Leakage and fusion of encapsulated drug/ molecules Liposomes help reduce the exposure of sensitive tissues to toxic drugs Production cost is high Site avoidance effect Fewer stables Flexibility to couple with site-specific ligands to achieve active targeting Akbarzadeh et al. Nanoscale Research Letters 2013, 8:102 Page 2 of 9 http://www.nanoscalereslett.com/content/8/1/102 one or bilayer membranes. The degree of drug encapsulation in the liposomes is influenced by the size and number of the bilayers, which is an important parameter in determining the circulation half-life of liposomes.[9] Based on their size and number of bilayers, liposomes can also be classified into one of two categories: (1) multilamellar vesicles (MLV) and (2) unilamellar vesicles. Unilamellar vesicles can also be classified into two categories: (1) large unilamellar vesicles (LUV) and (2) small unilamellar vesicles (SUV).[1] A single phospholipid bilayer sphere encloses the aqueous solution in unilamellar liposomes. Vesicles in multilamellar liposomes have an onion-like shape. Traditionally, a multilamellar structure of concentric phospholipid spheres

separated by layers of water will grow on the inside of numerous smaller unilamellar vesicles.[36] Different types of liposomes depending upon their size and number of lipid bilayer membranes are listed in Table No. 1.

Table 1: Types of liposomes based on structural parameters.

Vesicle Type	Abbreviations	Diameter Size	No. of Lipid Bilayer
Unilamellar vesicle	UV	All size range	One
Small Unilamellar vesicle	SUV	20-100 nm	One
Medium Unilamellar vesicle	MUV	More than 100 nm	One
Large Unilamellar vesicle	LUV	More than 100 nm	One
Giant Unilamellar vesicle	GUV	More than 1 micrometer	One
Oligolamellar vesicle	OLV	0.1-1 micrometer	Approx. 5
Multilamellar vesicle	MLV	More than 0.5	5-25
Multi vesicular vesicle	MV	More than 1 micrometer	Multi compartmental structure

Methods of liposome Production and Drug loading

Despite the wide range of conventional techniques utilized in liposome preparation, the techniques that are most frequently employed are the ones that include solvent injection, reverse phase evaporation, thin film hydration, and detergent removal (Karn et al., 2013; Meure et al., 2008). These methods involve the following basic stages: (i) lipids dissolved in organic solvents, (ii) removal of organic solvent, (iii) purifying and isolation of liposomes, and (iv) analysis of final liposomes.[37] The liposome preparation methods are shown in fig. 2.

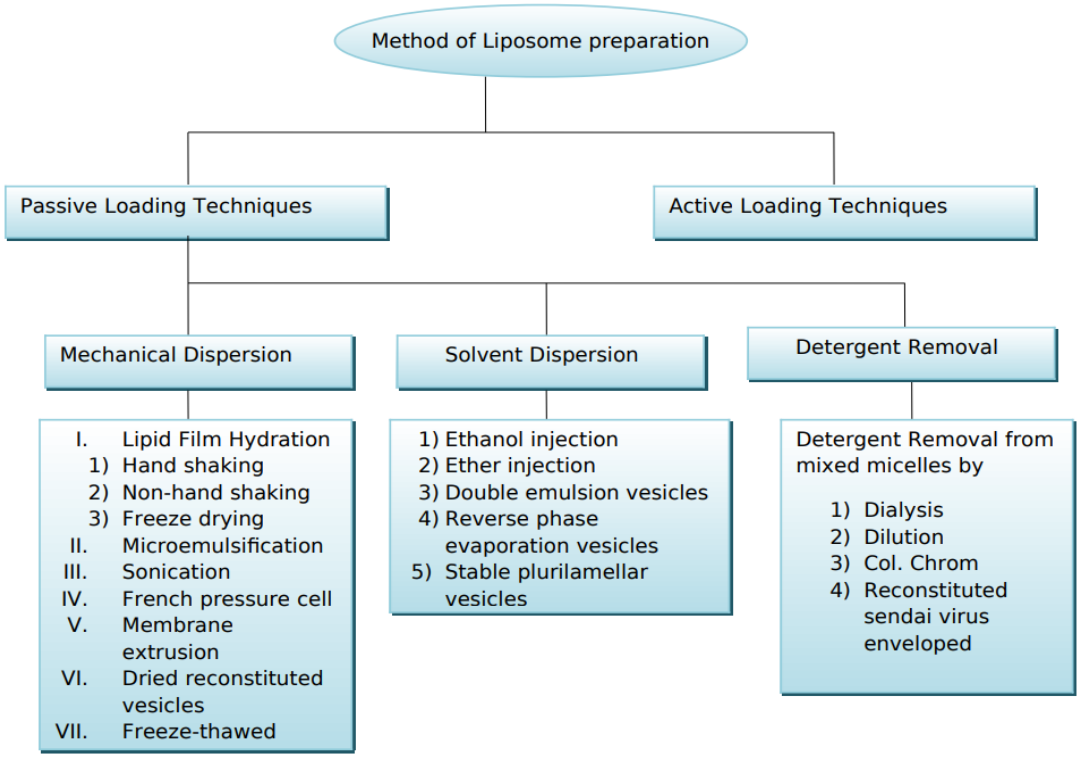

Fig. 2: Methods of liposome preparation.

Passive loading techniques

Mechanical dispersion method

This procedure involves dissolving a lipid into an organic solvent, dissolving the drug to be trapped in an aqueous solvent, and then hydrating the lipid phase while vigorously stirring. As a result of the aqueous phase's affinity for the polar head, the drug is subsequently trapped in liquid vesicles.

Thin film hydration using hand shaking or freeze drying method

The phospholipids are first dissolved in an organic solvent using this technique.[38] To fully mix the lipids, this method can provide the necessary clear solution, which is then poured into a flask with a round bottom and the solvent mixture extracted by rotational evaporation. Whenever the solvent evaporates under a vacuum, a thin, uniform film forms on the side of a round bottom flask at a temperature of 45 to 60 °C. Nitrogen gas is used in it to completely remove any remaining solvent.[39] Finally, the expanding lamellae are separated from the round bottom flask wall by agitation and dispersion, or hydration, of the lipid coating with an aqueous medium. At a temperature of 60–70°C, the hydration process lasts for about an hour. The liposomal suspension can be fully lipid hydrated by leaving it at 4°C overnight.[40] The

amount of medication solution that is loaded into multilamellar vesicles depends on how long the lipid film is hydrated and mixed. Low encapsulation, difficulties scaling up, and heterogeneous size distribution are drawbacks of this approach.[41]

Sonication

Small unilamellar vesicles are most frequently prepared with this technique. Multilamellar vesicles are solicited using a probe sonication in a passive environment or a bath-type sonicator.[42]

- **Probe sonication**

In which the liposome dispersion is completely covered by the solicitor's tip. Local heat is caused by a very high energy input into lipid dispersion. Titanium will slough off the pollutant from the solution using the probe sonicator.

- **Bath sonication**

The dispersion of liposomes in a cylinder is submerged in a sonic bath. The temperature may be easily controlled using this way. The substance to be sonicated may be kept safe in a sterile vessel distinct from the probe units or at an inert temperature.[43] With this technique, it is possible to regulate both the temperature of the ice bath and the product's temperature. One drawback of this approach is that the titanium probe's tip could accidentally communicate the product.

French pressure cell

Multilamellar vesicles are extruded using this technique through a tiny hole. By gently putting liposomes through a polycarbonate membrane filter with a predetermined pore size while under low pressure, the size of the liposomes is also decreased. Comparatively to small unilamellar vesicles created by sonication or detergent removal, French press vesicles tend to recollect contained solutes for a substantially longer period.

Comparing this approach to sonication offers several benefits. The resultant liposomes are a little bit bigger than SUVs from sonication. The method's disadvantages are that the high temperature is challenging to achieve and that the working quantities are very hard.[38]

Solvent dispersion method

In this solvent dispersion approach, the lipids are first dissolved in an organic solvent before coming into contact with an aqueous phase containing material that is contained in liposomes under rapid dilution and quick evaporation of the organic solvent.[44]

Ether injection

A solution of lipids is dissolved in dimethyl ether or ether/methanol. The material to be encapsulated's aqueous solution is then slowly injected with this solution mixture at a temperature between 55 and 65 degrees Celsius or low pressure. Liposomes are produced once the ether has been removed under a vacuum. This method's drawback is that it takes a lot of time and requires careful supervision while introducing the lipid solution.[45]

Ethanol injection

Rapid injection of an ethanol-lipid solution produces a large excess of the buffer. Instantaneously, the multilamellar vesicles develop. This is a straightforward procedure that involves progressively injecting an immiscible organic solution into an aqueous phase using a small needle at an organic solvent's vaporization temperature. Water is used to dilute the ethanol, and the phospholipid molecules are equally distributed throughout the mixture. This surgery is a significant percentage of SUVs.[45]

Reverse phase

A two-phase solution containing phospholipids in an organic solvent such as isopropyl ether, diethyl ether, or a combination of isopropyl ether and chloroform with an aqueous buffer is briefly sonicated to create the first water in oil emulsion. The excess organic solvent is eliminated when the pressure is lowered, which results in the creation of a viscous gel. By rotational evaporating the remaining solvent continuously while under lower pressure, liposomes are created. To encapsulate both small and large macromolecules, utilize this technique. The main drawback of the technique is the contact of the material to be encapsulated to organic solvents and to brief periods of proteins.[46]

Detergent solubilizing method

In this method of liposome synthesis, detergents that interact with phospholipid molecules bring the phospholipids into close contact with the aqueous phase. Serve to filter out from water the hydrophobic regions of molecules. The following methods are used to remove detergent from mixed micelles, including cholate, an alkyl glycoside, and Tritan X-100.[47]

Dialysis:

The detergents are used to solubilize lipids at their critical micelles concentrations. The micelles become progressively richer in phospholipid as the detergent is removed and finally combined to form large unilamellar vesicles. Detergent can be removed by dialysis. The main drawback of the method is the retention of traces of detergents within liposomes. Advantages of this are excellent reproducibility and production of liposomes population which are homogenous in size.[48]

Gel permission chromatography:

Detergent is eliminated via size-specific chromatography in gel permission chromatography. For gel filtration, sepharose 2B-6B, sephandexG-50, sephandexG-100, and sephacyl S200-S1000 can be utilized. Liposomes cannot enter the pores of beads that are stacked in a column. At modest flow rates, liposome extraction from detergent monomer is particularly effective.[49]

Dilution:

In this procedure, the micellar size and polydispersity substantially increase during the dilution of an aqueous mixed micelle solution of detergent and phospholipids with buffer, which causes the system to be diluted beyond the mixed micellar phase boundary. Monodisperse vesicles emerge spontaneously from polydisperse micelles.[50]

Active loading technique:

To show how amphiphilic amines, specifical catecholamine, are actively loaded into liposomes, the active drug encapsulation method was initially developed. In this method, after the creation of intact vesicles, some chemicals with the ionizable group and those with both lipid and water solubility can be inserted within liposomes.[51] To show how amphiphilic amines, specifical catecholamine, are actively loaded into liposomes, the active drug encapsulation method was initially developed. In this method, after the creation of intact vesicles, some chemicals with the ionizable group and those with both lipid and water solubility can be inserted within liposomes.[52]

Novel method:

As previously indicated, the flexibility to load medications with various features makes liposomes a good drug delivery technology.[53–55] Several variables, including EE, drug/lipid ratio, drug leakage and retention, sterility, production, and scale-up facility, cost-

effectiveness, and liposome stability, influence the choice of an effective method for drug encapsulation into liposomes.[54,55] Additionally, the type of drug, liposome composition, and liposomal production method are all factors that affect the amount of medicine that is encapsulated.[56] Passive and active techniques are the two different ways to encapsulate medicines into liposomes.[57] The process of encapsulating the medicine during liposome synthesis is known as the passive loading method. Hydrophilic drugs are diffused in the aqueous phase (both within and outside of liposomes), while those that are hydrophobic are found in the bilayer of the liposome.[54] In this process, liposomes can absorb the previously dissolved hydrophilic medication from the aqueous volume as they are being created. As a result, the concentration of the drug inside the aqueous core is comparable to the volume of aqueous solution that the liposomes contain. Due to several factors, including drug solubility, liposome size and charge, lipid concentration, and production process, the EE of medicines encapsulated by passive loading vary.[58] Ions and charged medicines cannot pass through the liposomal membrane. Otherwise, drug leakage could result from the uncharged medicines diffusing through the lipid membrane. Typically, this strategy yields poor EE, with high drug leakage for the medicines permeable to liposomal bilayer and a large volume of non-encapsulated drugs.[59] Active loading, as opposed to passive loading, can encapsulate hydrophilic medications with protonizable amine activities into liposomes, enhancing their EE.[57] As a result of the transmembrane pH or ion gradient created by the active loading principle, also known as distant loading, the medication is effectively driven through the lipid bilayer and can reach up to 100% loading in some cases.[60] This approach is used following liposome production. The gradient between the intact liposomes' already-formed inside and its exterior in the aqueous media, where the medication is dissolved.[61] Uncharged medicines that can cross the lipid membrane become protonated, which prevents them from diffusing out of the liposome and improves their EE and retention there.[62] When the drug is an amphipathic weak base (pKa 11) or weak acid (pKa > 3), the optimal loading efficiency is attained.[63] There are various methods for performing active loading, including the transmembrane gradient of ammonium sulfate for amphipathic weak bases, the gradient of calcium acetate for weakly acidic drugs, and the gradient of phosphate, the gradient of ethylenediaminetetraacetic acid (EDTA), and the method of ionophore loading.[62]

Characterization of liposomes:

After being created using various methods, liposomes are examined for their physical, chemical, and biological characteristics. To qualify, measure, and authorize the liposomes'

capability, characterization is needed. This affects how liposomes behave in living things. Size, size distribution (reported using the polydispersity index, PDI), surface charge (measured using a zeta potential), shape, lamellarity, phase behavior, EE, and in vitro drug release are the most studied properties to describe liposomes.[33] Analytical techniques used for the evaluation of liposomal properties are listed in Table No. 2.

Visual appearance:

The microscopic approach helps determine the size of big vesicles and uses bright-field, contrast, and fluorescent microscopes.[44]

Vesicle Size and Size distribution:

When administering liposomes via the parenteral route or inhalation. The size distribution is a fundamental characteristic.[64] Size and size distribution can be determined using a variety of techniques, the most popular of which is light scattering analysis.[65]

Familiarity:

Using Fraze Fracture Electron Microscopy and P-S1 Nuclear Magnetic Resonance Analysis, the similarity of the vesicle and the number of bilayers around the inner aqueous region of the lipid vesicles contained in liposomes is ascertained. The most crucial property of lipid vesicles is familiarity.[45]

Percentage Entrapment and Entrapment volume:

The amount of drug encapsulated in liposome vesicles is given by % entrapment. The minicolumn centrifugation method is mostly used to know the entrapment efficiency.

Entrapment volume (lit/mol) = 500/ 3.A.N.R

A = area of the membrane occupied by lipid

N = Avegadors number

R = Radius[66]

Surface charge:

By calculating the mobility of the liposomal dispersion in an appropriate buffer, a surface charge may be determined. Important factors affect liposomal Stability and Encapsulation effectiveness.[67]

Drug release:

Under dialysis circumstances, the in vitro drug release profile can be assessed. The dialysis bag membrane should be chosen following the requirements for each medicine. It shouldn't experience drug adsorption and must be freely permeable to the medication.[53] A specified molecular weight is removed from the liposomal sample before it is hermetically sealed inside the dialysis bag. The tubing membrane system is placed in a buffered saline solution with a pH of 7.4 to replicate natural fluid methods of release. To simulate an in vivo environment, the entire system is maintained at 37 °C and is constantly stirred. An aliquot of the sample is obtained at predetermined time intervals, and it is examined using the standard techniques for drug quantification. The number of samples must remain consistent. Consequently, an equivalent volume of new release medium is added once more to the system.[56,68] Mechanism of in vitro drug release can be performed by using the well-calibrated dialysis tube diffusion cell technique.[54]

Table 2: Analytical techniques used for the evaluation of liposomal properties.[1]

Characterization parameters	Instrument for analysis
Physical characterization	
Visual Appearance	Optical phase contrast microscope
Pyrogenicity	LAL test
Size distribution	Dynamic light scattering (DLS), Gel exclusion chromatography(GEC)
Concentration	HPLC, cholesterol oxidase assay
Liposomal stability	Stability chamber
Surface charge	Free flow electrophoresis, zeta potential measurement
Drug release	Diffusion
sterility	Culture test
pH	pH meter
Osmolarity	Osmometer
Phospholipid	UV absorbance, HPLC, GLC, TLC
Phase behavior	DSC, FFEM
Lamellarity	Spectroscopy, electron microscopy
Biological characterization	
Pyrogenicity	Rabbit fever response
Sterility	Aerobic/anaerobic culture
Animal toxicity	Monitoring survival rats
Chemical characterization	
Phospholipid concentration	HPLC/Barrlet assay
Anti-oxidant degradation	HPLC/TLC
Drug concentration	Assay method
Osmolarity	Osmometer
Phospholipid hydrolysis	HPLC/TLC

Phospholipid peroxidation	UV observance
Cholesterol concentration	HPLC/ cholesterol oxide assay
pH	pH meter
Cholesterol auto-oxidation	HPLC/TLC

Applications:

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.[69] Drug delivery uses liposomes because of their special characteristics. The hydrophobic barrier that surrounds an area of aqueous solution in a liposome prevents dissolved hydrophilic solutes from easily passing through the lipids.[70] A liposome can carry both hydrophobic and hydrophilic molecules by dissolving hydrophobic compounds into the membrane.[71] Various Liposomal products and their use are listed in Table 3.

Therapeutic application:

As a method for delivering a variety of medications, liposomes have shown encouraging outcomes. As a result, the thorough study of liposomes in medicine prompted researchers to create several liposomal formulations for the management and control of a wide range of disorders in addition to a wide range of therapeutic uses. Drugs are encapsulated inside liposomes, which improves their therapeutic impact due to changes in pharmacokinetics and pharmacodynamics.[72] The key elements to designing an effective liposomal formulation are the control of drug behavior in vivo and the reduction of drug toxicity in the organism. The therapy and identification of cancer are the primary uses of liposomes in clinical settings. The promise of liposomes for therapeutic uses is not restricted to the treatment of cancer, though. When it comes to research platforms, liposomes are said to be incredibly versatile.[73] This section will examine the state of the liposome market and how they are used, specifically, in the treatment of rheumatoid arthritis.[56]

Medicine and Pharmacology:

Therapeutic and diagnostic applications of liposomes in medicine and pharmacology can be distinguished. Liposomes are utilized as tools, models, or reagents in the basic research of cell connections, recognition processes, and the mechanisms of action of certain substances. Liposomes can include a variety of indicators or medications.[74] New applications for the transport of novel biotechnology products, such as antisense oligonucleotides, cloned genes, and recombinant proteins, are being made possible by advancements in liposome design.[75]

The majority of the literature discusses the possibility of creating liposomes from a variety of conventional drugs, which typically leads to increased therapeutic action and/or decreased toxicity when compared to the unbound drug. Since the pharmacokinetics of the liposomal medication can be altered, this enhances the bioavailability to the target cells that are present in circulation or, more significantly, to extravascular disease locations, such as tumors.[76]

Liposomes in parasitic diseases and infection:

Conventional liposomes are suitable delivery systems for therapeutic compounds that target these macrophages because they are digested by phagocytic cells in the body after intravenous administration. After being encapsulated in the form of liposomes with phosphatidylcholine, rifampin shows increased anti-TB activity.[57]

Genetic application:

Liposomes were investigated as a DNA delivery mechanism in 1979. Plasmid DNA must be multiplied by 100–1000 to transfer the information as effectively as a viral vector. The ability of liposomes to transfer genetic material, such as DNA fragments, to particular microorganism cells for peptide coding is exploited in this process. Clinical experiments for vaccines based on liposomes have yielded positive results, and additional human trials are currently underway.[1]

Diagnostic application:

For diagnostic purposes, liposomes are utilized as radiopharmaceuticals. Additionally, it has an immunodiagnostic agent property.[1]

Cosmetics and dermatology:

In the formulation for skin care, liposomes are utilized in the form of hydrogels or solutions. They are employed in the fields of dermatology and cosmetics due to their capacity to contain a wide variety of biological materials and deliver them to the epidermal cells. Liposomes are made topically from a variety of fatty substances and used to enclose or form spheres around cosmetic ingredients. They serve as a delivery mechanism.[77]

Table No. 3: Various liposomal Products and Their use.

Product	Drug	Use
Marquibo	Vincristine	Acute Lymphoblastic Leukaemia
Mika-some	Amikacin	Bacterial infection
Lipusu	Paclitaxel	Solid tumor

Inflexional	Inactivated hemagglutinin	Influenza
Abelcet	Amphotericin B	Fungal infection
Doxil	Doxorubicin	Tumor therapy
Diprivan	Propofol	Anesthesia
Amphoteric	Amphotericin B	Fungal infection
Daunoxome	Daunorubicin	AIDS-related Kaposi's sarcoma
Exparel	Bupivacaine	Pain management

Limitations of liposomes

In drug delivery applications, liposome stability is an important factor to take into account. The stability of the liposomes that contain medications determines their stability as well as how long they remain in the body and where they are distributed.[78] For the manufacturing, storage, and subsequent delivery processes of liposomes, stability is thought to be the primary concern.[53] The usual causes of liposome instability problems include drug leakage, aggregation formation, oxidation and/or hydrolysis of lipids, and liposomal fusion.[79] Finding a viable large-scale production process and an effective liposome sterilizing procedure are additional challenges for liposomal compositions.[56]

CONCLUSION

Researchers have been interested in liposome vesicles almost from their discovery in the 1960s and the demonstration of their trapping capability as prospective carriers of different bioactive compounds that may be exploited for therapeutic purposes in humans and animals. Their performance as drug delivery systems is influenced by a variety of circumstances. Since phospholipids have a lipophilic character, liposomes may penetrate the blood-brain barrier (BBB), allowing even hydrophilic medications (which ordinarily have difficulty doing so) to be designed as liposomes. With the ability of long circulation residence durations, liposomes with increased drug delivery to disease areas are increasingly gaining clinical recognition. Additionally, liposomes encourage the targeting of certain sick cells at the illness location. As the last point, liposomal medications are less toxic and nevertheless more effective than free alternatives. By gradually releasing the drug into the body, liposomes can prolong the effects of medications. The drug's distribution in the body is altered by the targeting option. They can also be utilized in vaccine formulation as an adjuvant. There are many ways to make liposomes, but the two that are most frequently used for the study are the film approach and the dehydration/rehydration method, among others. For the liposomal formulation to have the longest possible shelf life, liposome stabilization has been a problem. The particular binding is one of the recent liposome developments. Several commercial

liposomes have previously been found, registered, and successfully introduced in the pharmaceutical industry. Future marketing of more complex and well-stable liposomal compositions holds even more promise. Future advancements in vesicular systems, particularly in the treatment of cancer, will be made possible by the use of liposomal drug delivery systems.

ACKNOWLEDGMENT

The authors are grateful to the management of *Dadasaheb Balpande college of pharmacy, Besa, Nagpur-440034, India.* for providing necessary help to carry out this work.

Conflict of interests

The authors declare that they have no conflict of interests.

Abbreviations

EE: Encapsulation effectiveness, **PE:** phosphatidylethanolamine, **PC:** phosphatidylcholine, **PG**: Phosphatidyl glycerol, **PS:** Phosphatidylserine, **SM**: sphingomyelin, **MLV:** multilamellar vesicle, **LUV:** large unilamellar vesicle, **SUV:** small unilamellar vesicles, **EDTA**: ethylenediaminetetraacetic acid, P**DI:** polydispersity index, **BBB**: blood-brain barrier, **DNA**: Deoxyribose nucleic acid

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