ejpmr, 2022, 9(11), 294-307

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Review Article

ISSN 2394-3211

EJPMR

A REVIEW ON ADVANCES OF LIPOSOMES AS DRUG DELIVERY

Bhavin D. Pandya*, Shreyash A. Diwakar and Ajay K. Saluja

Department of Pharmaceutics, Krishna School of Pharmacy & Research, (Formerly, Babaria Institute of Pharmacy), Drs. Kiran and Pallavi Patel Global University, Krishna Edu Campus,Varnama, Vadodara, Gujarat, India.

***Corresponding Author: Bhavin D. Pandya**
Department of Pharmaceutics, Krishna School of Pharmacy & Research, (Formerly, Babaria Institute of Pharmacy), Drs. Kiran and Pallavi Patel Global University, Krishna Edu Campus,Varnama, Vadodara, Gujarat, India.

Article Received on 14/09/2022 **Article Revised on 04/10/2022** **Article Accepted on 24/10/2022**

ABSTRACT

Liposomes are spherical vesicles composed of one or more concentric phospholipid bilayers, surrounding an aqueous core that can be loaded with drugs. Liposomes represent a powerful new drug delivery system for entrapping both hydrophilic and hydrophobic drugs as non-toxicand biodegradable formulations. Overcame tissue uptake and increased biodistribution of the drug to target sites in vivo while minimizing systemic toxicity. This review provides a complete overview of liposome composition, properties, types, advantages, disadvantages, manufacturing processes, drug loading, characterization, applications and commercially available liposomal formulations.

KEYWORDS: Liposomes, Targeted drug delivery, Characterization, Applications, Marketed liposomes.

INTRODUCTION

Liposomes are small lipid-based vesicles composed of one or more lipid bilayers that facilitatethe encapsulation of hydrophilic, lipophilic, and amphipathic biologically active compounds.[5] Liposomes (or lipid vesicles) are a versatile platform for the delivery of drugs and other macromolecules into the human and animal body.[2] The name liposomes are derived from two Greek words, "Lipos" meaning fat and "Soma" meaning body. Liposomes can be formed in unilamellar or multilamellar structures of varying sizes and their name refers to their structural building blocks, phospholipids.[3] The major advantages of systemic liposomes as drug formulations derive from their biodegradability, reduced systemic toxicity, targeted delivery, protection of sensitive molecules and enhanced pharmacokinetic effects.[1] Compared with other colloidal delivery systems, liposomes offer the advantage of modifying their structural and physicochemical properties. Therefore, it is possible to modify the behavior of liposomes in-vivo and target them to specific sites in the organism.[4]

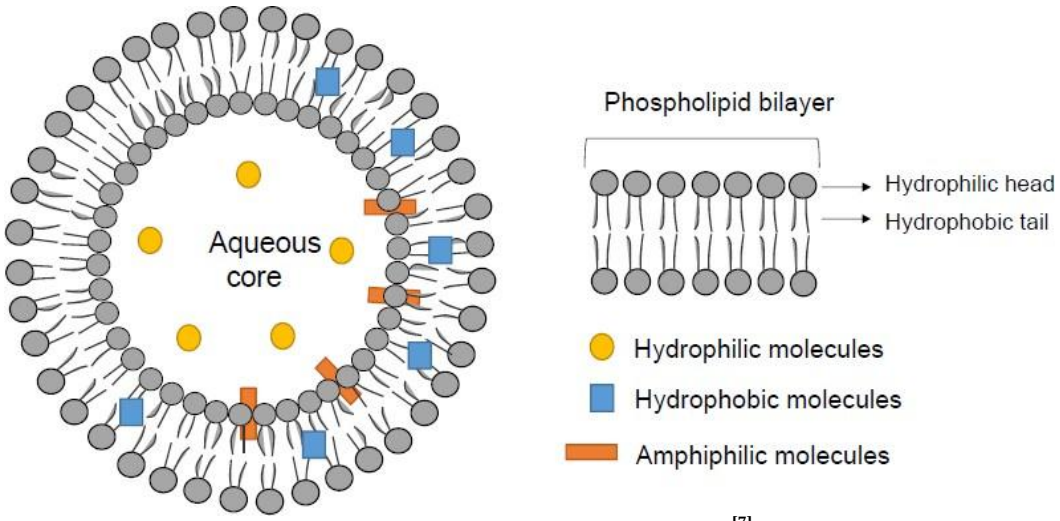


Fig. 1: General structure of liposome.[7]

COMPOSITION OF LIPOSOMES

Liposomes are mainly synthesized with cholesterol and various phospholipid components. Liposomes are primarily composed of phospholipids and include two

major categories including glycerophospholipids and sphingomyelin. Eukaryotic phospholipids are predominantly glycerophospholipids with a glycerol backbone. The chemical structure of glycerophospholipids consisted of a hydrophilic head group and a hydrophobic side chain.[1]

Phospholipids used in liposomes are phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG), and sphingomyelin. The total charge of liposomes depends on the charge of the phospholipid and can be either neutral, cationic, or anionic.[1]

Liposomes are spherical lipid bilayers 50–1000 nm in diameter that serve as convenient delivery vehicles for biologically active compounds. Topical application of liposomes has great potential in dermatological and anticancer drug delivery to reduce the toxic effects of drugs when administered alone or to increase drug circulation time and efficacy. Liposomes can be used to specifically target cells by attaching amino acid fragments such as antibodies or proteins, or appropriate fragments that target specific receptor sites. Improving the efficiency of DNA vaccination and gene therapy are just some of the future applications of liposomes. Liposomes are particularly effective in treating diseases that affect the phagocytes of the immune system, as they tend to accumulate in phagocytic cells. target. Phagocytes have provento be strange attackers.[3]

Liposomes have several structural and nonstructural components. The main structural parts of liposomes are:[3]

Phospholipids

Phospholipids are amphiphilic in nature. That is, it has a long hydrocarbon chain that is oil soluble and a hydrophilic head that is water soluble. In the presence of water, such lipids aggregate and self-assemble into various structures such as planar lipid bilayers, micelles and vesicles. Aggregate shape depends on lipid structure. Phospholipids are the major structural components of biological envelopes, and there are two types of phospholipids: phosphodiglycerides and sphingolipids. The most common phospholipid is the phosphatidylcholine (PC) molecule. Phosphatidylcholine particles are insoluble in water and aqueous media and are tightly aligned in planar bilayers to minimize adverse interactions between the main aqueous phase and long hydrocarbon-fat series. Glycerol containing phospholipids are mostly used component of liposomal formulations, accounting for more than 50% of the lipid weight of biological membranes. These are derivatives of phosphatidic acid. Examples of phospholipids include:[3]

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

Cholesterol

Cholesterol itself does not form bilayer structures, but can be incorporated into phospholipid membranes at very high concentrations, up to 1:1 or 2:1 molar ratios of cholesterol tophosphatidylcholine. Cholesterol is arranged in the membrane with its hydroxyl groups orientedtoward the aqueous surface and the aliphatic chains aligned parallel to the acyl chains at the center of the bilayer. Although the high solubility of cholesterol in phospholipid-liposomes has been attributed to both hydrophobicity and unique headgroup interactions, there is no clear evidence for cholesterol assembly in bilayers.[3]

Liposomes entrap DNA by one of two mechanisms that have come to be classified as either cationic liposomes or pH-sensitive liposomes. Cationic liposomes are positively chargedliposomes that interact with negatively charged DNA molecules to form stable complexes. The use of liposomes to transform or transfect DNA into host cells is known as lipofection. In addition to gene and drug delivery applications, liposomes can be used as carriers.[3]

PROPERTIES OF LIPOSOMES

Liposomes are used for drug delivery due to their unique properties. Liposomes encapsulate regions within an aqueous solution within a hydrophobic membrane. Dissolved hydrophilic solutes cannot readily increase lipids. Hydrophobic chemicals can dissolve into membranes, and liposomes can accommodate both hydrophobic and hydrophilic molecules. Liposomes are composed of bilayer structures intercalated via aqueous spaces.[3]

- They are water permeable.[3]
- They are sensitive to osmotic pressure. [3]
- There are positively charged membranes are impermeable to cations and negatively charged membranes are relatively permeable to anions. [3]

TYPES OF LIPOSOMES

Liposomes can be classified into five types according to their composition, function and intracellular delivery mechanism.[3]

1. Conventional liposomes
2. pH sensitive liposomes
3. Cationic liposomes
4. Immune liposomes
5. Long circulating liposomes

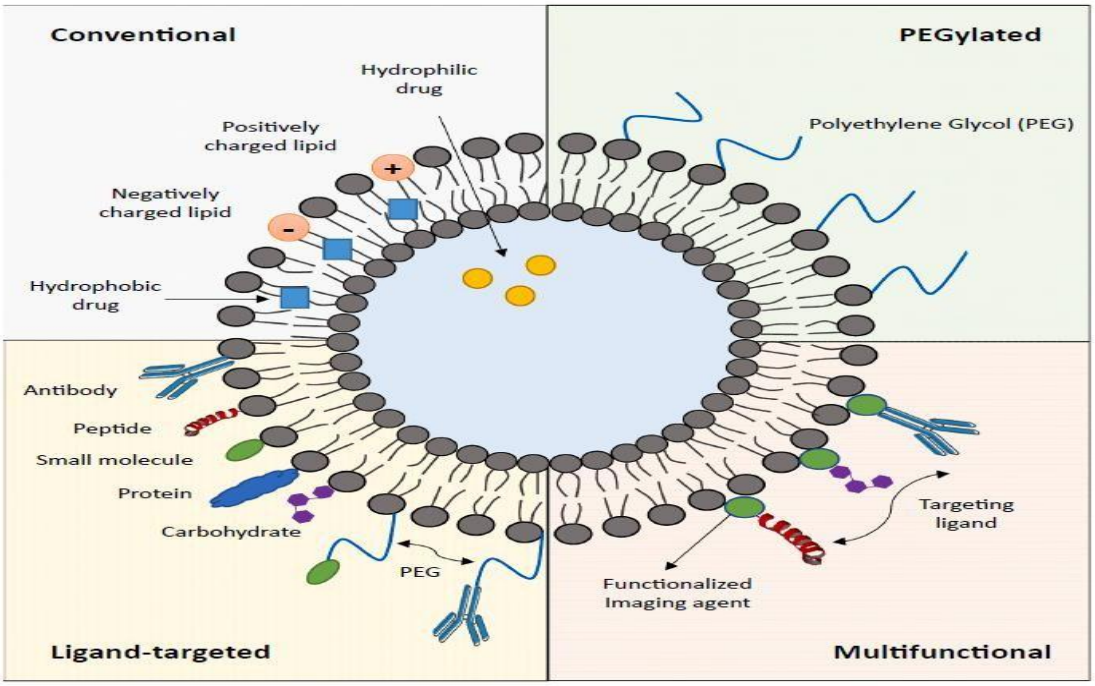


Fig. 2: Different types of liposomes depending on the composition, function and mechanism of intracellular delivery.[7]

1. **Conventional liposomes:** Conventional liposome-based mechanisms are the first generation of liposomes used in pharmaceutical applications. Conventional liposome formulations are mainly composed of natural phospholipids or lipids such as 1,2- distearolyl-sn-glycero-3-phosphatidylcholine (DSPC), sphingomyelin, egg phosphatidylcholine, and monosialoganglioside.[3]
2. **pH-sensitive liposomes:** Liposomes of varying composition can bind to a wide range of cell envelopes. For gene delivery, dioleoyl phosphatidyl ethanolamine (DOPE) has been recognized as the most efficient lipid for in vitro gene transfection of pH-sensitive liposomes or as a lipid helper for cationic liposomes. The function of phosphatidylethanolamine (PE) was thought to be that of a membrane synthesis promoter, as this lipid undergoes changes prior to acidification. After attachment to the cell surface, liposomes are taken up by endosomes, where they encounter a more acidic pH than external intermediates. Normally, the internal pH of endosomes is 6.5. Conventional pH-independent liposomes and their contents are delivered to lysosomes and stained. A final requirement for plasmid liposomes after cell penetration is to avoid accumulation in specific cellular compartments such as lysosomes. To address this deficiency, pH-sensitive liposomes have been proposed. pH-sensitive liposomes were developed based on the concept that viruses fuse with endosomal membranes and release their genetic material into the cytosol before reaching the lysosomes.[3]
3. **Cationic liposomes:** Generally, this is a simple procedure requiring the mixing of cationiclipids with the DNA and adding them to the cells. This results in the configuration of collectives composed of DNA and cationic lipids. The cationic lipid DOTMA was first synthesized and described by Feigner et al. (1987). It is presumed that complex formation simply results from ionic interactions between the positively charged head group of DOTMA and the negatively charged phosphate group of DNA. To determine the physicochemical properties of the composite, cationic lipids connected with DOPE and with various amounts of three different cationic surfactants have been examined by cryo-transmission electron microscopy. The results of the cryo-transmission electron microscopy analysis suggest that an excess of lipids in terms of charge leads to the entrapment of the DNA molecules between the lamellae in clusters of aggregated multilamellar structures. The option of surfactant use does not seem to influence the morphology of the DNA-lipid complexes.[3]
4. **Immune liposomes:** Another potential role of liposomes in medicine is to enhance the immune response by acting as an immunological adjuvant. Reconstitution of antigens into the liposome membrane or incorporation of antigens into the internal water core of liposomes enhances immune responses such as macrophage activation, antibody production, effective induction of cytotoxic cells, and subsequent anti-tumor activity. The advantages of liposomes as immune adjuvants are their biodegradability, low toxicity, low antigenicity, and the potential to target specific cells in-vivo. In fact, several data indicate that liposomes are excellent adjuvants to enhance immunogenicity against specific antigens, including glycolipids

(gangliosides), proteins, and antigens against pathogenic viruses. In vitro studies, usually performed in the absence of immunoglobulins, complement components, and macrophages, often yield very promising results, but in-vivo applications have been unsuccessful. Consider some additional potential targeting applications such as: B. Injection into various body cavities. Immunoliposomes are a viable option in immunoassays and diagnostic tests.[3]

5. **Long circulating liposomes:** Experimental results show that the liposomal matrix can change, but it can also change within a mononuclear phagocytic system that mainly involves intrahepatic uptake itself. Although the blood circulation time increased, the first significant improvement was achieved by incorporating GM1 ganglioside or phosphatidylinositol into the bilayer in an amount of 5-10% molar. The best results were obtained by replacing these two lipids with synthetic polymers containing lipids. Maximum cycle times were achieved using polyethylene glycol covalently bound to phospholipids. An average molecular weight of 1500-5000 Dalton seems to be optimal. It has been suggested that the presence of a steric barrier reduces the adhesion and adsorption (or at least adsorption with conformational changes) of blood components such as immunoglobulins, complementary proteins, fibronectin, and similar molecules that expose strange particles for uptake by macrophages.[3]

There are four types of liposomes depending on their lamellarity and size [3, 7]

1. SUV (Small Unilamellar Vesicles)
2. LUV (Large Unilamellar Vesicles)
3. MLV (Multi Lamellar Vesicles)
4. MVV (Multi Vesicular Vesicles)

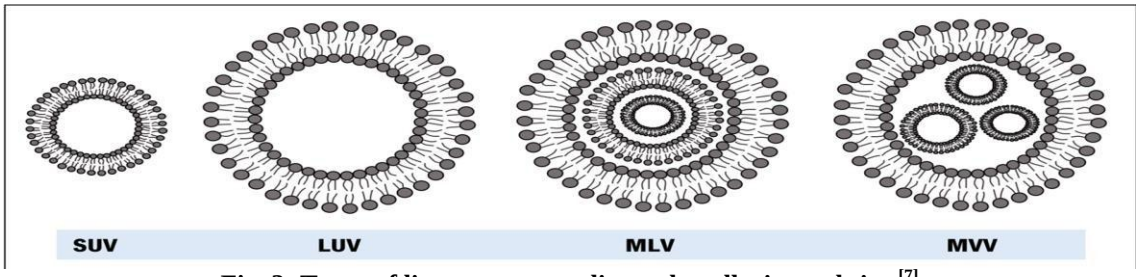


Fig. 3: Types of liposomes according to lamellarity and size.[7]

In addition to the above liposomes, liposomes such as immunoliposomes and stimuli- responsive liposomes have recently been developed.[3]

ADVANTAGES OF LIPOSOMES[3,4]

- Liposomes can complex both with negatively and positively charged molecules.
- Liposomes can carry large pieces of DNA and offer a degree of protection to the DNA from degradative processes.
- Liposomes can be targeted to specific cells or tissues.
- Liposomes increased efficacy and therapeutic index of drug (actinomycin-D).
- Liposome increased stability via encapsulation.
- Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, and nonimmunogenic for systemic and non-systemic administrations.
- Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, taxol).
- Liposomes help reduce the exposure of sensitive tissues to toxic drugs.
- Flexibility to couple with site-specific ligands to achieve active targeting.
- Site avoidance effect.

DISADVANTAGES OF LIPOSOMES[3, 4]

- Low solubility.
- Short half-life.
- Sometimes phospholipid undergoes oxidation and hydrolysis like reaction.
- Leakage and fusion of encapsulated drug/molecules.
- Production cost is high.
- Fewer stables.

METHODS FOR PREPARATION OF LIPOSOMES

The methods for preparation of liposomes can be divided in two main groups: active and passive loading methods. Active encapsulation includes methods that incorporate drugs after liposome preparation. They are mostly gradient loading techniques involving buffer or ammonium sulfate gradients. On the other hand, passive loading techniques include methods where a drug is encapsulated during the formation of liposomes. These include new and conventional liposomes preparation, which all consist of a few basic steps: the dissolution of lipids in an organic phase, subsequent dehydration of the organic phase, the dispersion of the lipid layer in an aqueous solution, and the purification and analysis of the resulting liposomes. They are still very popular and preferred because of their simplicity. However, they do not translate well in the scale-up of liposomes for industrial production.[5]

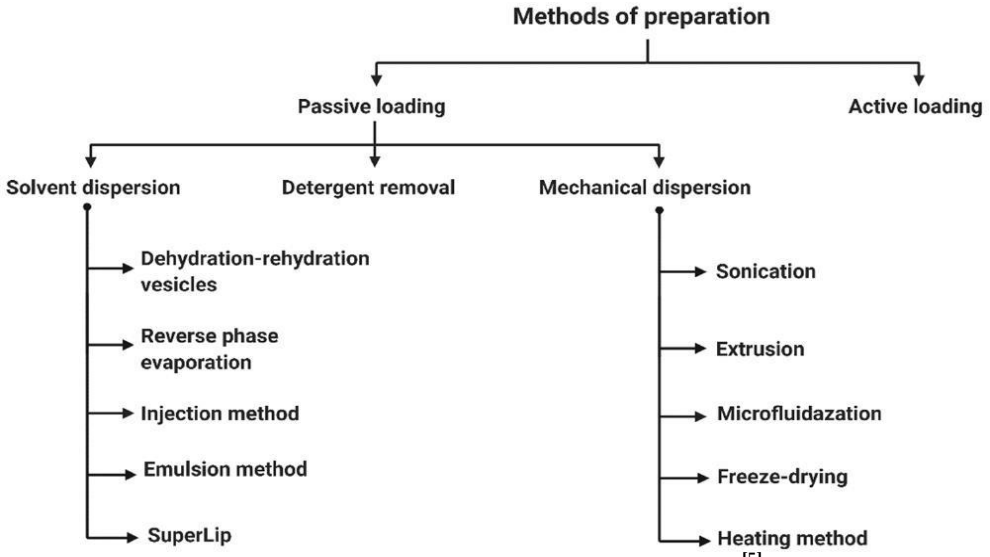


Fig. 4: Methods for preparation of liposomes.[5]

1. Passive Loading

1.1 Solvent dispersion methods

1.1.1 Dehydration–rehydration vesicles method: The thin film hydration method is one of the simplest and most used methods to prepare liposomes. It involves the dissolution of phospholipids in an organic solvent such as chloroform in a flask, followed by the evaporation of the solvent under high vacuum to form a thin lipid layer on the walls of the flask. The lipid film is subsequently rehydrated with an aqueous solution in which a hydrophilic drug of choice was previously dissolved and mechanical agitation is applied to detach the layer. Lipophilic drugs on the other hand are directly dissolved in the organic solvents along with the phospholipids. Phospholipids will self-assemble into bilayers around the solution, forming liposomes. Liposomes produced with this method are 10–100 m MLVs, not small enough for further applications, and as such it is usually combined with sonication or extrusion. This method includes the same first steps as the thin film hydration method; however, after the formation of liposomes, sonication or extrusion are applied to reduce the size of the vesicles. The obtained formulation is then freeze-dried, and the resulting powder is rehydrated when needed. Encapsulation efficiencies with those methods can go up to 90% with lipophilic compounds, but is around 10–30% with hydrophilic drugs. Encapsulation efficiency can be increased by the active drug loading technique. The major drawbacks of the methods are low entrapment efficiency, residual organic solvent and small- scale production.[5]

1.1.2 Reverse phase evaporation method: This method is carried out by dissolving phospholipids in an organic solvent (diethyl ether or isopropyl ether), adding small amounts of the aqueous phase in organic solvent, forming a water-in-oil emulsion and sonicating the solution to produce inverted micelles. This step is then followed by the removal of the organic solvent by rotary evaporation, generating a viscous gel. The gel later collapses and the excess phospholipids in the environment form a second layer around micelles, resulting in the formation of liposomes. The contact between the organic solvent and the drug to be encapsulated can degrade fragile molecules such as peptides, making reverse phase evaporation method unsuitable for their encapsulation. The large aqueous space created in liposomes with this method enables the encapsulation of macromolecules, with encapsulation efficiency going up to 90% with some compounds.[5]

1.1.3 Injection method: In this method, phospholipids are dissolved in ethanol or ether and the lipid solution is injected dropwise with a needle into an aqueous solution containing a hydrophilic drug. Unilamellar liposomal vesicles are then formed. The major drawbacks of this technique include its time-consuming process, a high organic residue in the final suspension, and difficulty removing ethanol since it forms an azeotropic mixture with water. Furthermore, an evaporation could damage the structure of vesicles and the particle size distribution is difficult to control with this method. Additionally, since lipids are poorly soluble in ethanol, ethanol injection often leads to the formation of heterogeneous liposomes. encapsulation efficiency reported for the injection method is around 20% for hydrophilic compounds and greater than 60% for some lipophilic compounds. The recently developed inkjet method is a modern variation of the injection method that offers excellent control of particle size and potential for scaling up. An inkjet printer is used to inject a phospholipids solution into water, generating SUVs (20–100 nm) with higher encapsulation efficiency than thin film hydration and high reproducibility.[5]

1.1.4 Emulsion method: For this method phospholipids are dissolved in a water/organic solvent mixture, forming a water-in-oil (w/o) emulsion. The mixture is then transferred into a large aqueous solution and vigorously agitated, producing a double emulsion, a water-in- oil-in-

water emulsion (w/o/w). A second lipid monolayer surrounds the particle, forming droplets with an aqueous core and a lipid bilayer containing residual organic solvent. The organic solvent can be removed by passing some nitrogen through the double emulsion, resulting in the formation of unilamellar liposomes with high encapsulation efficiency. An advantage of this technique includes the control over liposomes' size by agitation and the amount of lipids used.[5]

1.1.5 SuperLip method: Supercritical assisted liposomes formation (SuperLip) is an emerging alternative to traditional techniques. Unlike other methods, this process begins with the creation of water droplets through a spray atomization. The droplets are passed into a high-pressure vessel filled with phospholipids dissolved in an expanded liquid (ethanol and carbon dioxide). The droplets are quickly surrounded by phospholipids, creating lipid vesicles. Liposomes aqueous suspensions are collected from the bottom of the vessel. The expanded liquid is then removed from the top of the vessel, and depressurized to separate carbon dioxide from ethanol. The advantages of the method are its simple continuous operative layout not requiring numerous steps, its lower solvent residue and its high reproducibility and encapsulation efficiency (up to 99% for hydrophilic compounds and 87% for lipophilic compounds). This method shows potential for long circulating drug delivery systems, obtention of Nanometric dimensions, minimal solvent residue, high Encapsulation efficiencies, and no variations between batch production as compared with the previously described methods.[5]

1.2 Detergent removal method: In this method, detergent-lipids micelles are formed by either hydrating phospholipids with a solution of detergent or drying phospholipids and detergent from an organic solution and hydrating them with an aqueous solution. The detergent is then removed from the micellar solution by dilution, dialysis, column chromatography, or adsorption, resulting in the formation of liposomes. This method is, however, time-consuming, leads to low Encapsulation efficiency of hydrophobic drugs, detergent residues in the formulations, and even methods employed for the removal of detergent can also remove small hydrophilic compounds.[5]

1.3 Mechanical methods: These methods are often used for post-formation processing of liposomes to disrupt LUVs and modify their size, lamellarity, or homogeneity to ultimatelyform SUVs.[5]

1.3.1 Sonication: This is the most widely used method for the preparation of SUVs. MLVs are sonicated in a bath sonicator or with a probe sonicator. In the former, the liposome dispersion in a container is placed into a bath sonicator, which allows for a better control of the temperature and the protection of liposomes in a sterile vessel. On the other hand, in a probe sonication, the tip of the sonicator is directly inserted into the liposome dispersion, where the energy input is very high and results in local heat. There is also a risk of metal sloughing off and contaminating the solution. The main inconvenience of this method includes low internal volume, possible degradation of drugs and phospholipids, possible contamination (probe sonication), and a heterogeneous mix of MLVs and SUVs.[5]

1.3.2 Membrane Extrusion: The extrusion method involves the size reduction of previously prepared liposomes by passing them through polycarbonate filters of different pore size (depending on the application) with repeated cycles of extrusion under moderate-high pressure. The process can be time-consuming.[5]

1.3.3 Microfluidization method: In this process, a lipid solution in organic solvent is injected into a central feeding channel while aqueous solutions are added to side channels, which intersect with the main channel at the center. As the lipid solution passes between two aqueous streams in the microfluidic channel, mixing occurs and thus monodisperse nanometric liposomes are obtained in the outer channels. The size of the liposomes can be controlled by adjusting the flow conditions, so no size reduction is needed post preparation. Microfluidization suffers from high solvent residues in liposome suspensions and a difficulty to reach high production scale. Additionally, the production cost of the micro-circuit used in this method is higher than other conventional methods.[5]

1.3.4 Freeze Drying method

The freeze-drying method is the most popular technique used to stabilize liposomes and extend their storage. It involves freezing liposomes-cryoprotectant mixtures at -40^0C for some time and then drying them at the same temperature for 48 hours, followed by drying of the product at room temperature. Upon the addition of an aqueous solution, the lyophilized powder spontaneously forms spherical lipid vesicles. Sucrose and glycerol (cryoprotectants) were shown to stabilize liposomes during this process by preventing the aggregation and fusion of mini-domains. Liposomes made with this method have an average size of 100–300 nm and an encapsulation efficiency of 40–60%.[5]

1.3.5 Heating method: In this method, phospholipids are added to an aqueous solution containing glycerol (3% v/v) at a bath temperature up to 120^0C. The use of glycerol is motivated by its solubility in water and its ability to act as an isotonic agent, increasing the stability of liposomes. It is also a physiologically acceptable material which does not need to be removed from the final product, reducing toxicity of liposomes. Materials can be encapsulated at any step of the process except for heat-sensitive compounds (e.g., DNA), which should be added at ambient temperature, after liposome

preparation. Liposomes produced with this technique have nanometric sizes, long term stability (12–14 months), andlow EE that can however go up to 81% with DNA. The main advantage of this technique isthat it does not use any organic solvent for the preparation of liposomes. Additionally, the use of heat to prepare liposomes abrogates the need to carry out a sterilization stepafterwards.[5]

2. Active loading: Active loading or remote drug loading works with drugs that are poorly soluble and able to go from uncharged species (freely diffusible across the lipid membrane) to charged ones (not freely diffusible across the lipid membrane), and it is therefore limited to amphipathic weak acids or bases. Non-amphipathic drugs can however be chemically modified in some cases with cyclodextrins, for instance. The driving force of this process is the transmembrane gradient of ions, as they can be exchanged with amphipathic drugs inside liposomes. This gradient is created by concentrations of $(NH_4)_2SO_4$ or $Ca(C_2H_3O_2)_2$ in the liposomes, which are 1000-fold greater than concentrations outside the liposome. Indeed, remote loading uses an ammonium sulphate gradient to encapsulate weak bases into liposomes or a calcium acetate gradient for weak acids. The un-ionized drug-base or drug-acid outside the liposomes crosses the liposomal membrane where it is trappedinside by its ionization and the formation of an insoluble salt with the intraliposomal counter ion. A pH gradient can also be used for this method. Active encapsulation usually results in high EE and enables controlled drug release.[5]

All the methods of liposomes preparation involve four basic steps:[4]

1. Drying lipids through the evaporation of the organic solvent.
2. The lipid dispersion in an aqueous media.
3. Liposome purification.
4. Analysis of the final product.

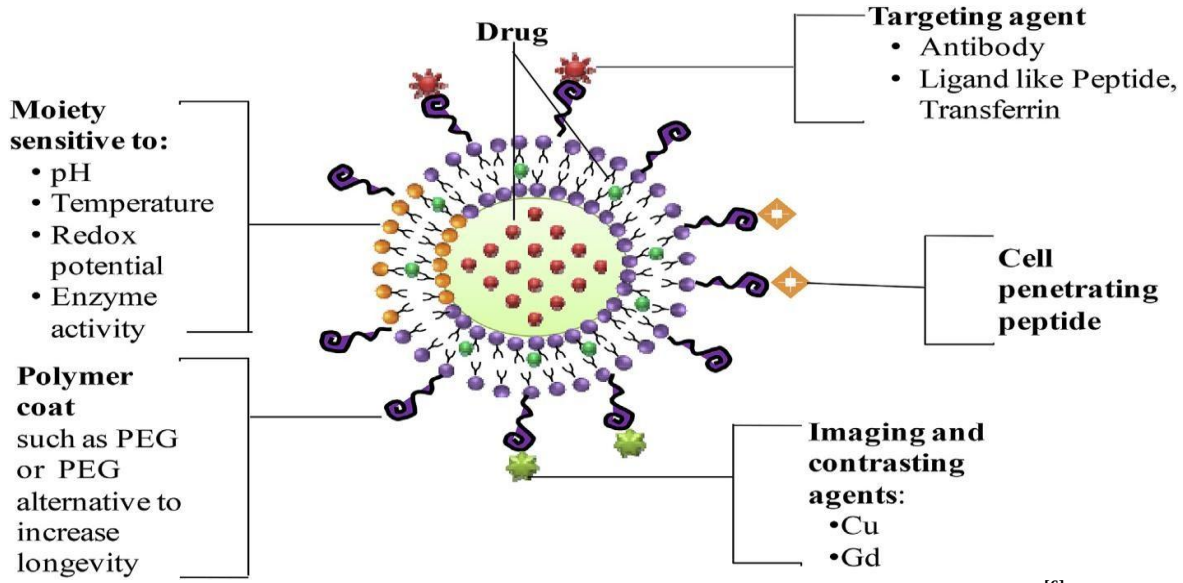


Fig. 5: Modification potential of liposomes for targeting, diagnosis and treatment.[6]

CHARACTERIZATION OF LIPOSOMES

The properties of liposomes are highly dependent on their size, lamellarity, surface charges, and encapsulation efficiency. Therefore, an accurate estimation of those parameters is essential. The concentration of drugs encapsulated can be determined using SpectraMax M5 plate reader, which measures the absorbances and standard curve of known concentrations.[5]

1. Particle size and Polydispersity index: The size and polydispersity index of liposomes are the most relevant features in liposome characterization. It has known that the liposome size shown to be a crucial factor for inhalation and parenteral administrations and to determine the circulation half-life of liposomes. While small liposomes can circulate in the organism for long time, large liposomes are more quickly eliminated from the blood circulation. For drug delivery, the desirable size of liposomes usually ranges between 50 and200 nm. The polydispersity index value reveals in terms of size, the degree of sample heterogeneity, that can be monodisperse or polydisperse. polydispersity index can bedimensionless and scaled such that values range from 0 to 1. In drug delivery applications using liposomes, a polydispersity index value equal or below 0.3 indicates an acceptable and homogenous liposomal population, whereas high polydispersity index value is associated with a very broad size distribution (heterogeneity) or even several liposomal populations in the sample. The calculation of polydispersity index is based on the particle size, refractive index of the solvent, the measurement angle and the variance of the distribution. The most used technique to measure these two features is dynamic light scattering also known as photon

correlation spectroscopy. Dynamic light scattering analyses the continuous motion of the dispersed particles in solution (Brownian motion), resulting in scattering of the incident light. The scattering of the light is correlated with the diffusion level of the liposomes in suspension (small particles diffused faster than the large particles). The evaluation of mean size is calculated based on the amount of light scattered. Dynamic light scattering is considered a simple, easy, fast and reliable method with the capacity to evaluate the liposome size in their native environment. Extensive range of measurement ability from a few nanometers to several micrometers is also applied. However, this technique has some limitations, involving the difficult to differentiate single particles from aggregates and the high sensitivity to detect low number of impurities. Recently, a size characterization tool called nanoparticle tracking analysis was introduced to determine the size by measurement of the diffusion coefficient of particles in a sample. Dynamic light scattering is used to determine the diffusion coefficient of particles based on the reads of the intensity change of scattered light. Whereas, nanoparticle tracking analysis find the diffusion coefficient by the movements of individual particles in successive optical video image. Nanoparticle trackinganalysis can be a good approach to verify the size determined by dynamic light scattering due they measure the same physical property. Therefore, the size measured by nanoparticle tracking analysis should be like that observed in dynamic light scattering technique. The capacity of nanoparticle tracking analysis to simultaneously measure size and particle scattering intensity, besides allowing to distinguish particles of different refractive index within the same sample solution, makes a direct estimation of particle concentration. Dynamic light scattering is the most used method to determine size of liposomes in the sub-micron range by using light scattering from a laser that passes through liposomal solution to analyze the intensity of scattered light as a function of time. The Brownian motion of the particles correlates with their hydrodynamic diameter, which affects their scattering capacity. Dynamic light scattering can also be used to study the stability of formulations over time, as particle sizes increase when the particles agglomerate, a sign of instability. However, it does not provide accurate measurements for highly polydisperse samples and assumes a spherical shape for the liposomes. Alternatively, electron microscopy techniques are powerful tools to study the size, size distribution, lamellarity, and shape of liposomes. They are, however, expensive, time consuming, as the analysis is done manually and therefore prone to human error. The combination of those two methods overcomes most of their shortcomings as stand-alone techniques and can provide a more accurate estimation of liposomal parameters. Asymmetric field flow fractionation has been increasingly used in recent years to measure the size distribution of polydisperse liposomal samples in the sub- micron range. Unfortunately, the high cost associated with the technique limits its widespread application. Finally, laser diffraction and nanoparticle tracking analysis are commonly used, along with the novel multispectral advanced nanoparticle tracking analysis.[5, 7]

2. Lamellarity: Cryo-transmission Electron Microscopy is the most used method and provide useful information regarding liposome lamellarity such as their bilayer thickness and inter-bilayer distance. Lamellarity is also estimated with ^{31}P NMR (nuclear magnetic resonance), where the degree of lamellarity is determined from the signal ratio before and after Mn^{2+} addition. This method is very dependent on Mn^{2+} and buffer concentrations and the type of liposome analyzed. Small angle X-ray scattering (SAXS) and electron microscopy are also used to determine lamellarity.[5, 7]

3. Zeta Potential: Zeta potential is an important factor used to measure surface charge of liposomes, which strongly affects their stability, pharmacokinetics properties, and affinity with entrapped drugs. For instance, charged liposomes are less prone to aggregation while negatively charged liposomes improve stability and aggregation and are less prone to phagocytosis than positively charged ones. Zeta potential is measured by the electrophoretic mobility of charged particles once an electric field is applied. It reflects the potential difference between the electric double layer of mobile particles and the layer of dispersant around them. Adsorption of ions onto liposomes' surfaces can affect their zeta potential andeven their sign.[5]

4. Encapsulation efficiency: Encapsulation efficiency is defined as the percentage of drug successfully entrapped into liposomes with regards to the initial amount of drug used. Encapsulation efficiency mostly depends on phospholipids compositions, lipids to drug ratio, and the methods of preparation. Various analytical methods like Ultraviolet-visible spectroscopy, high-pressure liquid chromatography, gas chromatography, gas chromatography-mass spectroscopy, and even quantitative NMR can be used to determine the concentration of drug inside the liposomes, depending on the nature of the active compound. In the study, Peretz and co-workers used a cryo-electron microscopy technique to determine the exact concentration of drug encapsulated per liposome by calculating the trapped volume of individual liposomes using their micrographs. This method can prove extremely useful in the development of liposomal pharmaceutics.[5]

5. Phase behaviour: The phase behaviour of liposomes can be represented by the critical temperature (Tc) is an important parameter that can affect the fluidity of the lipid bilayer. For drug delivery applications, phase behavior is highly considered due the fact that the lipid bilayer permeability to entrapped hydrophilic drugs increases with lipid membrane fluidity. Several other liposomal properties including fusion, aggregation, stability and protein binding are also dependent on the

phase behavior of a liposomal membrane. Usually, the most common method used for study and determination of the Tc is the differential scanning calorimetry. This thermal analysis technique is based on the evaluation of differences in heat flow, between a sample reference and a study sample. Both samples are subjected to a programmed heating, cooling or isothermal treatment using a meticulous control of the atmosphere, typically saturated with nitrogen gas. The Tc can be also measured by Thermogravimetry analysis, fluorescence probe polarization, electron paramagnetic resonance, Nuclear magnetic resonance, Fourier transform infra-red and X-ray diffraction. To calculate the phase behavior of phospholipids in lipid bilayers can be also explored the molecular dynamics simulations.[7]

6. In-vitro drug release: The evaluation of the in-vitro drug release profile can be performed using dialysis conditions. The selection of dialysis bag membrane should be in accordance with the drug specifications. It must be freely permeable to the drug and should not occur drug adsorption. Liposomal sample is placed into the dialysis bag with specific molecular weight cut-off, hermetically tied. The tubing membrane system is put into a simulated physiological fluid means release medium, usually a buffered saline at pH 7.4.

The full system is kept at 37^0C to mimic an in vivo environment, and under continuous stirring. At defined time points, an aliquot of sample is taken and analyzed by the conventional methods used for drug quantification. The volume of samples needs to keep constant. Thus, an equal volume of fresh release medium is placed again in the system. The data are used to establish the release profile by plotting the cumulative release percentage against the select time points. As extrapolation to in-vivo performance of liposomes as drug delivery system, the results obtained from the in-vitro release study are widely considered in the development of liposomes for the controlled release of drugs.[7]

STABILIZATION OF LIPOSOMAL FORMULATIONS

The main parameters affecting liposomes stability are size, composition, and electric charge. Stability issues are also caused by chemical hydrolysis of acyl ester bonds and oxidation of the polyunsaturated acyl chains, leading to drug leakage, fusion, and aggregation of liposomes. Cholesterol plays an essential role in stabilizing liposomes and minimizing phospholipids exchange. It prolongs their circulation time by several hours. In addition, cholesterol is incorporated into phospholipids bilayers because it was shown to increase separation between the choline head groups and reduce the hydrogen bonding strengths and electrostatic interactions, thus making the lipid bilayer more stable and lowering its permeability to water and other molecules. Polymers have been extensively used to stabilize liposomes, especially polyethylene glycol (PEG). PEG is indeed known to improve stability of liposomes and to enhance their circulation time by creating a protective hydrophilic layer on the surface of liposomes, hampering binding with opsonin protein and serum protein- mediated lipid elimination. It is also possible to increase stability through cross-linking the hydrophobic tail groups of lipids within lipid bilayers or the functionalized headgroups of adjacent lipid bilayer of multilamellar liposomes. Since the aqueous environment in which liposomes exist is propitious to the stability issues previously mentioned, freeze drying offers a possibility to overcome these issues. Freeze-drying, discussed above, is one of the most used post-processing techniques to increase the stability of liposomes, as it eliminates water molecules and prevents the degradation of sensitive substances. However, it is necessary to develop new stabilization methods that do not involve surface modifications of liposomes in their surface properties and prevent unwanted bio-interference interactions. Chen and co- workers offer a new method to stabilize liposomes using a stiff nano bowl in the aqueous layer. Indeed, for the first time they developed liposomal doxorubicin supported by nano bowls that show reduced drug leakage, improved drug delivery to tumor sites, and increased killing of tumor cells compared to conventional liposomes. Similar methods that aim to support or stabilize the aqueous layer instead of the lipid layer should be explored in the future as they could improve drug loading and reduce leakage.[5]

The software based screening and key optimization of critical quality attributes and process parameters are being employed for the development of safe, stable and economic liposomes. The modern approach uses the design of experiments for optimization to bring precise development of liposomal products at industrial outset. To prepare liposomes for in-vivo purpose, it is essential to prepare them with optimum in-vivo kinetics and good throughput conditions of liposome compositions. Formulation by design could be a potential strategy to identify desired liposome compositions through high throughput screening methods, the design and optimization of critical factors for the development of liposomes. Several optimization techniques such as Factorial designs, Box-Behnken designs, Fractional Factorial designs, Plackett-Burman designs have been used for the preparation of efficient liposomal drug delivery systems.[6]

SCALE-UP OF LIPOSOMAL FORMULATIONS

The production of liposomes on a large scale in the pharmaceutical industry can be arduous, limiting their translation from bench to bedside. This is because of the limited feasibility of scaling most conventional bench-scale techniques to clinical scale production and the multi- step process of manufacturing them. Other issues with scale-up include multi-step processes, non-uniformity in size distribution of the liposomes from batch to batch, exposure to high concentrations of organic solvents (degrade biological molecules), and low

reproducibility. Therefore, new rapid, cost-effective, scalable manufacturing processes with reduced steps are needed. Microfluidics has been reported to be a cost-effective, scale-independent technique to include in the production process, offering high-throughput, continuous production with good reproducibility and particle size control. It can eliminate the need for lipid hydration and extrusion since the vesicles are formed and hydrated in the microfluidic chamber. Roces and co-workers demonstrated the production of SUVs PEGylated tumor-targeting liposomes with a high encapsulation efficiency (>90%) using microfluidization. Furthermore, in a new technique called the nanoprecipitation/antisolvent technique, phospholipids were dissolved in a biocompatible solvent along with a stabilizer to spontaneously form sub-micron vesicles upon exposure to water. When optimized, this technology could eliminate the need for an extrusion and a filtration step, avoiding the use of organic solvents altogether. There are still some challenges and issues of liposomes scale- up production that should be addressed, including oxidative degradation of liposomes, validation of depyrogenation of raw lipid materials, complete removal of residual traces of organic solvents, and batch uniformity.[5]

ADMINISTRATION AND DISTRIBUTION OF LIPOSOMES

Liposomes can be administered via different routes such as oral, topical, nasal, transdermal routes and in the brain owing to their versatility. They are chemically degraded and excreted through their uptake and clearance by the reticuloendothelial system. Liposomes modify tissue distribution and the clearance of the loaded drugs. The physicochemical properties of the liposomes formed, such as the lipid composition, the surface charge, size, and route of administration, directly affect the pharmacokinetics of the liposomal drug. The main sites of accumulation of liposomes are tumor sites, liver, and spleen. It is important to note that the pharmacokinetics of the liposome-drug depends on the carrier and not the encapsulated drug until the drug is released. Once in the bloodstream, plasma proteins, such as opsonins will help the reticuloendothelial system recognize and eliminate liposomes. This causes lipid transfers and rearrangements in liposomes, which induces liposome breakdown and rapid release of the cargo to the plasma. The encapsulated drug will then interact with its target cells. Finally, liposomes are eliminated by the target tissues after recognition by the reticuloendothelial system at the hepatic level and metabolized by Kupffer cells. They can also be metabolized by macrophages in the spleen.[5]

MECHANISM OF ACTION FOR DRUG DELIVERY FROM LIPOSOMES

Liposomes can deliver their content into through various mechanisms including lipid exchange, surface interactions, fusion, endocytosis and pinocytosis.[5]

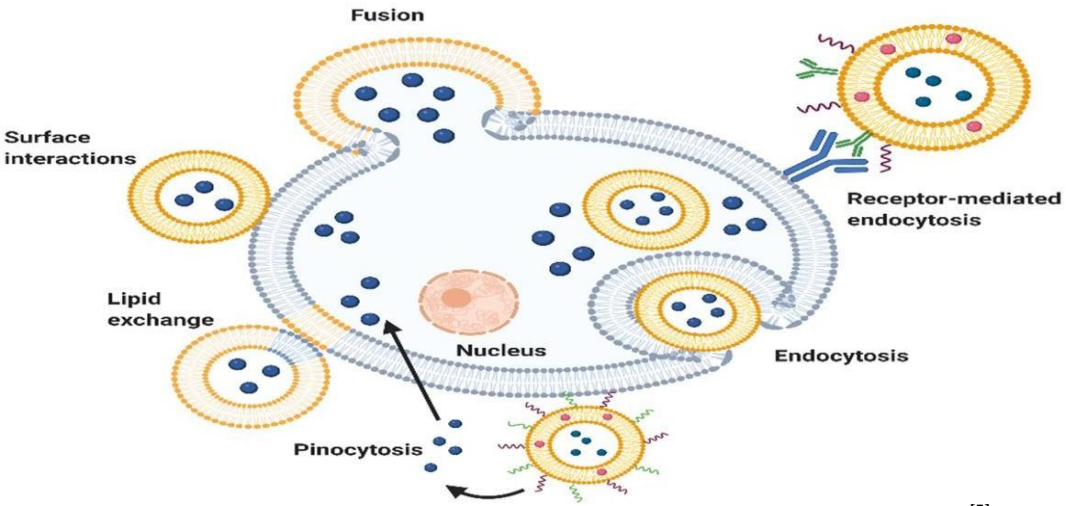


Fig. 6: Mechanism of action for drug delivery from liposomes uptake into cells.[5]

With the recent advent of surface-modified liposomes, it is possible to decorate the external surface of liposomes with various materials such as peptides, antibodies, polymers, pH sensitive compounds, etc. Those liposomes can have specific bindings to cell receptors that create long-lasting drug release. Long-circulating liposomes can be prepared by coating their surface with polyethylene glycol. Polyethylene glycol was indeed reported to increase liposomes stability, distribution in target tissues and their half-lives. Additionally, pH sensitive liposomes can direct the accumulation and the release of their content at a specific target site, such as cancer cells, which have been shown to be slightly acidic pH 6.4 to 6.8 vs. normal pH 7.4. The design of pH sensitive liposomes can be achieved with pH labile linkages, pH cleavable crosslinking, or by adding charge shifting polymers at their surface. The similarity of liposomes with cell membranes and their small sizes makes them particularly useful for intracellular drug delivery. Liposomes can in fact interact with cells and deliver their material by four main mechanisms. These are: (a) specific interactions with cell-surface components, (b) endocytosis

by phagocytic cells, (c) fusion with cell membrane by insertion of lipids from liposomes into plasma membrane, followed by the release of liposomal content into the cytoplasm and (d) exchange of bilayer components of liposomes with cell membrane components. More than one of these mechanisms can be operating at the same time. Neutral, anionic, and stimuli-responsive liposomes are internalized into the cells by endocytosis, releasing their contents into the cytoplasm. On the other hand, cationicliposomes are reported to use membrane fusion and endocytosis to deliver drugs to cells. PEGylated liposomes gradually release their content into the extracellular fluid, which thenenters cells either via diffusion or pinocytosis. Finally, ligand-targeted liposomes enter the cell via receptor-mediated endocytosis where ligands are removed and liposomes release their drug content. To overcome the fast elimination problem, immunoliposomes (surface modified liposomes) programmed to be digested by macrophages, which then transferred their contents to target tissues, exploiting the immune response) show good promise.[5]

TARGETING OF LIPOSOMES

For liposomes to be successful as drug carriers, they must be selective in delivering drugs tovarious tumours. There are mainly two types of targeting of liposomes.[8]

1. Active targeting
2. Passive targeting

1. Active targeting: The strategy used here is to target the drug to cancer cells or tumourtissues with minimum accumulation in healthy cells or tissues, in turn causing higher efficacy and minimum toxicity. Active targeting is achieved by grafting specific ligandson the surface of the liposome. The choice of targeted ligands is based on the regulated receptor on tumour tissue. The ligand bearing liposomes require specific interactions of ligands with receptors present on the target cells, followed by the uptake of encapsulated cargo by receptor-mediated endocytosis, which is made with biorender.com. In some reports, the ligand targeted liposomes showed enhanced efficacy.[8]

2. Passive targeting: When liposomes get targeted by the natural distribution system to certain organs (liver and spleen) and tissues (tumour and cancer cells), the phenomenonis known as passive targeting. In the cases of tumours, drug-loaded liposomes are knownto accumulate in the tumour area due to the leaky vasculature Enhanced Permeability and Retention effect (non-targeted liposomes) which makes the basis for cancer therapy by passive targeting. This effect can be used to differentiate between normal and cancerous tissues as the capillaries in the tumour area possess increased permeability. In addition, besides permeability, there are several features including porosity and pore size of tumour which vary with the type and stage of the tumour and influence the passive targeting effect.[8]

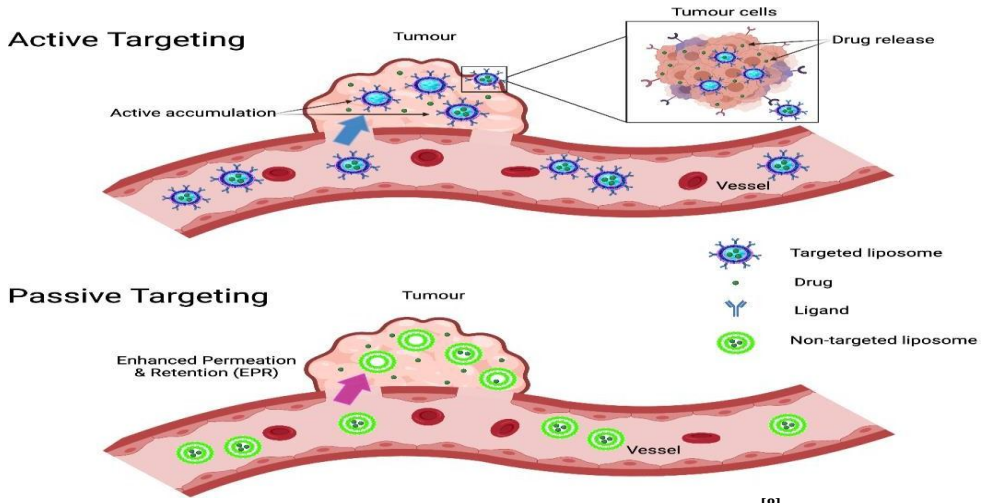


Fig. 7: Active and passive targeting of liposomes.[8]

APPLICATIONS OF LIPOSOMAL DRUG DELIVERY

The liposomal formulations have been developed to treat various diseases over several advantages compared to free drugs or other delivery systems. Due to their excellent drug encapsulation, which enhances the therapeutic efficacy of the drugs, reduced toxicity and reduced degradation of drugs, a high biocompatibility because they are made of naturally occurring phospholipids, increased therapeutic index of drugs, direct targeting of specific tissues or receptors, bypass multidrug resistance observed in cancer therapy and in antimicrobial therapy exhibited by bacteria. The possible versatility of liposomes makes their applications in various fields. Liposomes have been successfully developed for parenteral, transdermal and oral drug delivery in treatment of eye disorders, respiratory disorders, brain targeting, vaccine adjuvants, antimicrobial drug delivery, cancer therapies and gene delivery.[5]

1. Liposomes in diagnosis: Peptide targeting liposomes tagged with imaging agents can be used to efficiently deliver diagnostic agents to targeted sites.

They can be used for diagnostic purposes to detect several diseases, including cancer. Song and co-workers constructed new dual targeted paramagnetic liposomes for cancer diagnostic, with $\alpha_v\beta 3$- integrin and neuropilin 1 receptors on the surface and the magnetic resonance imaging contrast agent Gd-DTPA inside the liposomes. The liposomal complexes were able to efficiently bind to tumor tissue and could potentially improve the effect of magnetic resonance imaging contrast agents for tumor-specific imaging early on. A recent study developed an approach in which reagent-loaded liposomes are used to effectively diagnose a SARS-CoV-2 gene target, even in cases missed by real time-polymerase chain reaction (RT-PCR). This is a great tool much needed to support coronavirus disease-2019 detection.[5]

2. Liposomes for brain targeting: There is a crucial need to enhance treatment of central nervous system (CNS) diseases such as multiple sclerosis, stroke, neurodegeneration,and brain tumours. CNS drug delivery has not been completely efficient mainly because of the blood–brain barrier, which serves as a selective semipermeable layer causing low drug permeability. Due to their nature, liposomes have shown great promise in delivering therapeutics to the brain. Cationic liposomes are the most effective for drug delivery to the brain, probably because of their electrostatic interactions with the negatively charged cell membranes, which enhances nanoparticle uptake. However, due to their non-specific uptakeand their binding to serum proteins, large amounts are needed for a therapeutic effect, whichcan be toxic. It is necessary to develop stimuli-sensitive liposomes and liposomes with bettertargeting of specific brain areas.[5]

3. Liposomes as vaccine adjuvants: Cationic liposomes are more potent carriers for vaccines, because they induce stronger immune reactions at low dose. Antigen–liposomal complexes can increase and maintain exposure of the antigen in lymph nodes, making for an enhanced uptake by immature phagocytic antigen-presenting cells such as macrophages anddendritic cells. Recently, the global pandemic caused by the respiratory illness designated coronavirus disease-2019 created an urgent international need for vaccines. Moderna developed a pegylated liposomal mRNA vaccine which encapsulates nucleoside modified mRNA that encodes the SARS–CoV–19 spikes, glycoprotein, with 94.1% effectiveness in a Phase 3 trial. The vaccine is now widely administered in the USA and Canada for the prevention of COVID–19 since December 2020, with mild to moderate side effects reported in general. At present vaccines for SARS–CoV–19 by the pharmaceutical companies, Moderna and Pfizer utilize lipid nanoparticle systems for encapsulating messenger RNA (mRNA) to enhance their stability and performance. Both have been approved (for emergency use) and are currently being used to vaccinate people in several countries. It is essential to mention here that the mRNA molecules are extremely delicate and fragile and cannot withstand the enzymes both in laboratory conditions as well as in our bodies. The negative charge of mRNA electrostatically repulses the anionic cell membrane and hinders its entry into the cell and hence a delivery vehicle is needed. The development of these vaccines can be owed to the decades of development of liposomes/lipid nanoparticles research.[5, 8]

4. Liposomes in eye disease: The development of ophthalmic drug delivery systems is very challenging because of the various ocular protection mechanisms against foreign substances, impeding drug entry (tear dilution, lacrimation). The eye can be divided into an anterior segment composed of structures such as the cornea, conjunctiva, iris, ciliary body, the lens and aqueous humour, and the posterior segment made of the retina, optic nerve, choroid, and vitreous humour. Some of the pathologies that can affect the anterior segment are dry eye disease, cataracts, conjunctivitis, and keratitis, while the posterior segment is affected by diseases causing significant damages in vision (irreversible blindness, glaucoma and diabetic retinopathy). López-Cano and co-workers offer an extremely detailed review of liposomal formulations in the treatment of various ocular diseases. Liposomes are good forophthalmic drug delivery because they decrease rapid clearance from the vitreous cavity, enhance intravitreal half-life of drugs, and reduce toxicity associated with higher dosage.[5]

5. Liposomes in cancer therapy: Cancer is a serious health problem in the world. Chemotherapeutic agents used for cancer treatment usually cause undesired side effects because of their harmful effects on normal, noncancerous tissues. Additionally, cancer drugs do not reach all cancerous cells, which can lead to metastasis and drug resistance. Liposomes, on the other hand, can dispense large quantities of drugs to tumours while avoiding off targets and elimination by the mononuclear phagocyte system, making treatment more effective. Peptide functionalized liposomes can be used to target several selective receptors overexpressed on cancer cells. The tumor cells tend to have a more acidic environment which can be exploited for targeted drug delivery, using pH sensitive liposomes. Doxil® (or Caelyx® in Europe) was the first Food and Drug Administration approved PEGylated liposomal doxorubicin for the treatment of various cancers.[5]

For example, curcumin loaded liposomes: The encapsulation of partially water-soluble drugs in liposomes enhances their bioavailability resulting in enhanced stability and antitumor activity, but reduced unwanted effects. Curcumin is the herbal polyphenol compound which possesses remarkable anticancer potential in pancreatic adenocarcinoma. Various liposomal preparations loaded with curcumin were developed and investigated for different physicochemical parameters and stability at 4°C and 37°C in human plasma in vitro. The most efficient formulation amongst all with respect to these parameters was found to be PEGylated one. The formulation scientists emphasize on

the development of safe, stable andeconomical liposomal drug delivery systems with enhanced efficacy for tumor targeting and capability to carry a broad range of anticancer agents with favorable outcomes.[6]

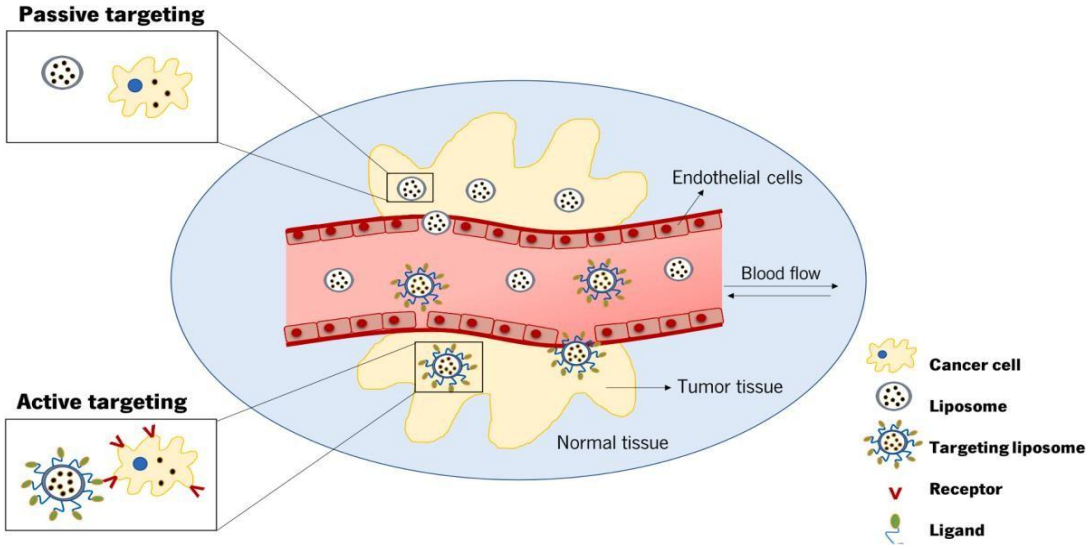
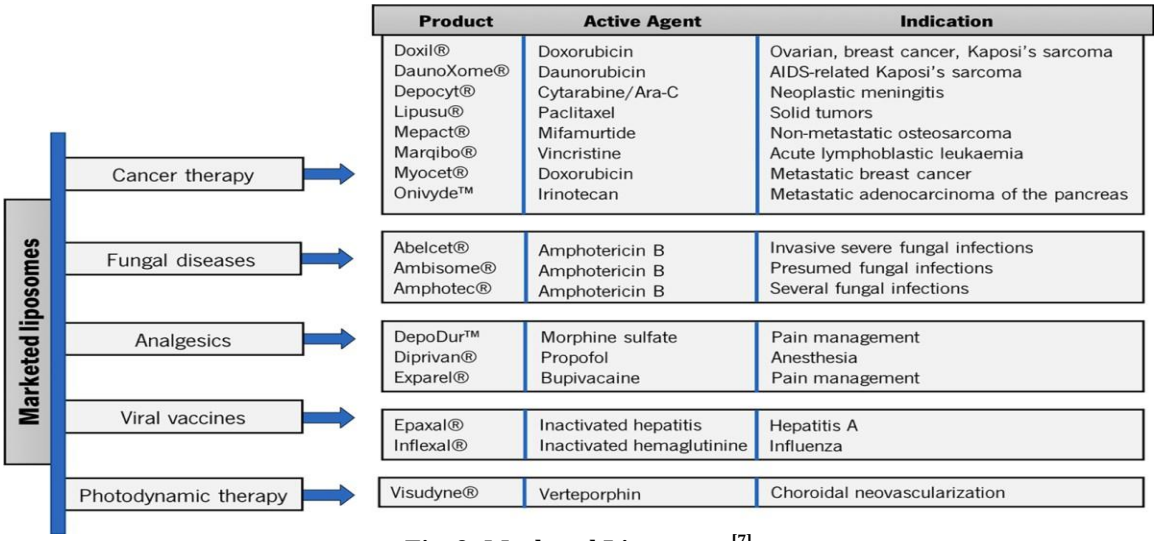


Fig. 8: Schematic representation of passive and active targeting of liposomes on atumor tissue for increasing the therapeutic efficacy of drugs.[7]

6. Liposomes as delivery systems for antibiotics and anti-infectives: The multidrugresistance in bacteria is a crucial problem. By using various resistance mechanisms by the bacteria such as biofilms formation, reduced membrane permeability, antibiotic-modifying enzymes, and efflux pumps. By considering, liposomes are particularly useful to potentiate the activity of antibiotics and bypass resistance mechanisms, while reducing associated toxicity. Previous studies have demonstrated that liposomal antibiotics have increased activity against various resistant bacteria. Indeed, Rukavina et al. developed azithromycin loaded liposomes that improved the activity of antibiotics against skin infections caused by methicillin resistant staphylococcus aureus. The formulation also retained azithromycin in the skin more efficiently and prevented biofilm formation, with a minimum biofilm formation concentration 32-fold lower than the free drug. In a recent study, Ribeiro and co-workers showed the improved in vitro antimicrobial activity of their hybrid liposomal formulation composed of pectin, liposomes and norfloxacin against multidrug resistant strains of Pseudomonas aeruginosa, E. coli, Salmonella sp., and Campylobacter jejuni. Thepectin was used for its resistance to low pH and its ability to interact with mucin. It is believed to increase the antibiotic's bioavailability after oral administration. Additionally, an in vivo chicken embryo study confirmed the safety of the formulation. Finally, in the treatment of pulmonary P. aeruginosa infections in cystic fibrosis patients, inhaled liposomaltobramycin is the drug of choice. Many studies reported its efficacy against the pathogen, along with an enhanced bacterial killing at reduced doses.[5]

MARKETED LIPOSOME PREPARATIONS



Product	Active Agent	Indication
Doxil®	Doxorubicin	Ovarian, breast cancer, Kaposi's sarcoma
DaunoXome®	Daunorubicin	AIDS-related Kaposi's sarcoma
Depocyt®	Cytarabine/Ara-C	Neoplastic meningitis
Lipusu®	Paclitaxel	Solid tumors
Mepact®	Mifamurtide	Non-metastatic osteosarcoma
Marqibo®	Vincristine	Acute lymphoblastic leukaemia
Myocet®	Doxorubicin	Metastatic breast cancer
Onivyde™	Irinotecan	Metastatic adenocarcinoma of the pancreas
Abelcet®	Amphotericin B	Invasive severe fungal infections
Ambisome®	Amphotericin B	Presumed fungal infections
Amphotec®	Amphotericin B	Several fungal infections
DepoDur™	Morphine sulfate	Pain management
Diprivan®	Propofol	Anesthesia
Exparel®	Bupivacaine	Pain management
Epaxal®	Inactivated hepatitis	Hepatitis A
Inflexal®	Inactivated hemaglutinine	Influenza
Visudyne®	Verteporphin	Choroidal neovascularization

Fig. 9: Marketed Liposomes.[7]

CONCLUSION

Liposomes have shown great promising targeted drug delivery with several approved liposomal drugs on the market. With distinct properties like biocompatibility, biodegradability, accompanied by their nano size, liposomes have potential applications in diverse areas such as the delivery of antibiotics, anti-fungal, anti-cancer drugs, genetic medicines and imaging. It is very essential to acquire a comprehensive perception about the physiological and pathophysiological variations existing in patients to examine the effects of the administered liposomal delivery system. It would help in the development of efficient liposomes for personalized treatment of the diseases. Advancements in liposomal drug delivery require consistent effort to improve the frontiers of knowledge in design and manufacturing, assessment of toxicology, cellular interactions and clinical evaluation. Presently, several liposomal drugs are clinically approved and commercially available, while many more formulations are either being investigated in different stages of clinical trials orawaiting approval.

REFERENCES

1. Ahmed KS, Hussein SA, Ali AH, Korma SA, Lipeng Q, Jinghua C. "Liposome: composition, characterisation, preparation and recent innovation in clinical applications." Journal of Drug Targeting, 2018; 1–20.
2. Has C, Sunthar P. "A comprehensive review on recent preparation techniques of liposomes." Journal of Liposome Research, 2019; 1–30.
3. Daraee H, Etemadi A, Kouhi M, Alimirzalu S, Akbarzadeh A. "Application of liposomes in medicine and drug delivery." Artificial Cells, Nanomedicine, and Biotechnology, 2016; 44: 381–391.
4. Akbarzadeh A, Sadabady RR, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati KK. "Liposome: classification, preparation and applications." Nanoscale Research Letters, 2013; 8: 102–110.
5. Gbian DL, Omri A. "Lipid-Based Drug Delivery Systems for Diseases Managements." Biomedicines, 2022; 10: 1–16.
6. Saraf S, Jain A, Tiwari A, Verma A, Panda PK, Jain SK. "Advances in liposomal drug delivery to cancer: An overview." Journal of Drug Delivery Science and Technology, 2020; 56: 1–14.
7. Guimaraes D, Paulo AC, Nogueira E. "Design of liposomes as drug delivery system for therapeutic applications." International Journal of Pharmaceutics, 2021; 601: 1–15.
8. Agarwal K. "Liposome Assisted Drug Delivery: An Updated Review." Indian J Pharm Sci, 2022; 84(4): 797–811.