Journal of Exploratory Research in Pharmacology **2023** vol. 000(000) | 000–000 Epub
DOI: 10.14218/JERP.2023.00002

Review Article

Medical Application of Liposomes

Biswajit Basu[1], Bhupendra Prajapati[2*], Ayon Dutta[3] and Himanshu Paliwal[2]

[1]Department of Pharmaceutical Technology, School of Medical Sciences, Adamas University, Barasat, Kolkata, West Bengal, India; [2]Shree S K Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva, Mahesana, India; [3]Department of Pharmaceutical Technology, Brainware University, West Bengal, India

Received: January 12, 2023 | **Revised:** May 08, 2023 | **Accepted:** June 20, 2023 | **Published online:** September 04, 2023

Abstract

Liposomes are a potential drug delivery system involving encapsulation into a phospholipid-based vesicular carrier system. In comparison with conventional delivery systems, liposomes may be advantageous due to site-specific targeting, controlled release patterns, enhance stability, and reduce associated toxicity, among other processes. Several researchers in the last decade have attempted to develop simple or modified forms of liposomes for the effective delivery of various types of therapeutic agents. This review is focused on a discussion about some of the recent literature on the medical application of liposomes. An account of the mode of action of different types of liposomes as a supporting basis for their superior therapeutic efficiency was provided. The application of liposomes in the delivery of anticancer, anti-bacterial, and anti-fungal drugs, among others, was discussed. Along with this discussion, liposomal carriers for the management of diseases related to the respiratory and nervous system were included along with a special emphasis on the outcomes of current literature. The information gathered through this review will be useful in furnishing ideas about the current status of research studies conducted on the formulation and development of liposomal carriers.

Introduction

The words "lipo" and "soma," which signify fat and body respectively, are the roots of the term liposome. Further, the word "liposome" refers to the phospholipids that make up its structural components rather than its size, and it may be produced in different dimensions utilising any unilamellar or multilamellar design. Dr Alec D. Bangham, a British haematologist, identified liposomes for the first time at the Babraham Laboratory in Cambridge in 1964. In the experiment, a negative stain was applied to dried phospholipids to test the institute's newest electron microscope. A tiny bubble known as a liposome is constructed from the same substance as a cellular membrane (vesicle). To cure cancer and other diseases, medications can be encapsulated inside liposomes. Typically, phospholipids comprise a head portion as well as a tail portion for making membranes. The head repels water while the tail comprises a lengthy hydrocarbon chain that attracts it. Phospholipids are present in stable two-layer membranes that are seen in nature (a bilayer). When there is water present, the heads align to form a surface because the hydrocarbon chains are attracted to it.[1] The tails align to produce a surface far from the water since water repels them. An additional layer of heads has been drawn there by the water within. When there is only one bilayer around the aqueous core, large or small unilamellar vesicles can be detected; however, there are often massive multilamellar vesicles when there are several concentric bilayers. Monolayer and bilayer structures are referred to as micelles and liposomes, respectively. Liposomes are employed for the delivery of therapeutic agents because of their distinct characteristics. As such, they can consist of a broad range of components to maximise the amount of medication carried by each particle by safeguarding encapsulating chemicals from metabolic processes. Traditional liposomes have a significant drawback in that they swiftly clear the circulation because plasma proteins are adsorbable in their "second generation state," known as sterically stabilised liposomes. A coating process, typically a lipid variant of polyethylene glycol (PEG), demonstrated several advantages through *in vivo* pharmacokinetic properties, making them excellent components for anticancer drug delivery systems. To achieve additional desired results, the chemical makeup of the lipid bilayer can also be altered. For example, long-circulating liposomes can construct a composite with nucleic acids to influence gene delivery or regulate gene expression, with the capacity to de-

Keywords: Liposomes; Phospholipid; Targeted delivery; Controlled release; Stability; Cancer therapy.

Abbreviations: AMB, amphotericin B; CHP, cholesterol pullulan; DNA, deoxyribonucleic acid; DOPE, di-oleoyl phosphatidyl ethanolamine; LET, liposomal encapsulation technology; MLV, multilamellar vesicles liposomes; MP, macrophage; PC, phosphatidylcholine.

***Correspondence to:** Bhupendra Prajapati, Shree S K Patel College of Pharmaceutical Education and Research, Ganpat University, Kherva 384002, Mahesana, India. ORCID: https://orcid.org/0000-0001-8242-4541. Tel: +91 9429225025, E-mail: bhupen27@gmail.com

How to cite this article: Basu B, Prajapati B, Dutta A, Paliwal H. Medical Application of Liposomes. *J Explor Res Pharmacol* 2023;000(000):000–000. doi: 10.14218/JERP.2023.00002.

liver components to the cytosol by employing endosomal pathways for drug delivery.[2] The inherent potential of conventional and stealth liposomes to promote the localisation of anticancer medicines to solid tumours is another intriguing characteristic of such liposomes. The higher tumour selectivity of liposomal medications compared to free pharmaceuticals is the result of the differential accumulation of liposomal drugs in tumour cells versus normal tissues. Liposomes have been used as effective drug carriers for a variety of substances, including medicines, poisons, proteins (peptides), enzymes, antigens (antibodies), and nucleotides. Liposomes have been regarded as excellent models of cell membranes.[3] Liposomes can be targeted in many ways. This includes "passive targeting" through a technique for bulk recognition. In such instances, targeting is accomplished by modifying the affinity, charge densities, fluidity, and size, as well as other aspects of the carrier. To accomplish "active targeting," a different methodology, namely a molecular recognition approach is employed. Making a direct and precise connection between a membrane receptor of the specific cell and a particular recognition region on the surface, which generates molecular recognition, allows for the targeting of specific cells. After being injected into the body, a passive targeting mechanism is primarily responsible for the primary tissue distribution. However, an active targeting mechanism takes control when there is a van der Waals distance between the liposome and the target cell. Active targeting is therefore highly significant and more advantageous than passive targeting.[4] It is possible for liposomes to inadvertently or purposefully cling to the surface of the cell during the early phase of liposome-cell interaction. Selective adsorption involves an interaction between both sides of the cell and the liposome, as opposed to nonspecific adsorption, which only requires an electrostatic or hydrophobic link between the cell and the liposome.[5]

Liposome structural components

The globular lipid bilayers known as liposomes, which range in size from 50 to 1,000 nm, are useful delivery systems for substances with biological activity. Liposomes can be applied topically in dermatology to administer anticancer therapies that reduce the toxicity of the treatments when given alone or to extend the drugs' half-life and increase their efficacy. By affixing relevant amino acid fragments that target certain receptor sites or antibodies, proteins, or other appropriate pieces, liposomes may be utilised to precisely target cells.[6] Liposomes are made up of both structural and nonstructural parts. Liposomes' principal structural components are listed below.

Phospholipid

The primary structural elements of biological coverings are phospholipids, of which there are two types: Phosphodiglycerides and Sphingolipids. The phosphatidylcholine (PC) molecule is the most prevalent phospholipid. Due to their insoluble nature in water and aqueous conditions, phosphatidylcholine particles arrange themselves tightly to lessen the detrimental contact between the long hydrocarbon fatty chain and the bulk aqueous phase in planar bilayer sheets. Glycerols, especially phospholipids, which weigh up to over half of the lipid in the membranes, are included in the majority of liposome formulations.[5,7]

Cholesterol

Without producing a bilayer structure on its own, cholesterol may be present in membranes in extremely higher quantities; for instance, a 1:1 or even 2:1 molar ratio of cholesterol to phosphatidylcholine. In the membrane, cholesterol is located in the centre of the bilayer, parallel to the acyl chains, wherein the hydroxyl group faces the aqueous region. Although interactions between hydrophobic and specified head groups have been associated also with high solubility of cholesterol in phospholipid liposomes, it is unknown how cholesterol is organised in the bilayer.[8]

Classification of liposomes

Conventionally developed liposomes

The first invention of liposomes to be employed in medicinal applications is the traditional liposome-based method. The majority of natural phospholipids or lipids used in conventional liposome formulations include Sphingomyelin, egg phosphatidylcholine, 1,2-distearoryl-sn-glycero-3-phosphatidyl choline (DSPC), and monosialoganglioside.[9]

Liposomes sensitive to pH

Different types of liposomes can strongly attach to cell membranes. Di oleoyl phosphatidyl ethanolamine (DOPE) has long been known to be the most efficient lipid for cationic liposomes or as a lipid helper in pH-sensitive liposomes during *in vitro* gene transfection in terms of gene transport. Since this lipid changes before acidification, it has been theorised that phosphatidylethanolamine promotes membrane formation in its natural state. Liposomes are internalised into endosomes after adhering to the cell surface, where they come into contact with a more acidic pH. Typically, the inner pH of endosomes is 6.50.[10] The transfer of conventional, pH-insensitive liposomes to lysosomes results in the disintegrate of the liposomes. The ultimate criterion for plasmid liposomes is just to circumvent accumulating in specific cell divisions like lysosomes after cell penetration. To avoid this, pH-sensitive liposomes have demonstrated usefulness. The idea that viruses might connect with the endosomal membrane and transmit the genetic information to the cytosol before penetrating the lysosomes led to the invention of pH-sensitive liposomes.[11]

Cationic liposome

Adding cationic lipids to the cells after combining them with DNA is often a straightforward process. As a result, collectives made up of DNA and cationic lipids are formed. The cationic liposomes firstly synthesise and characterise the cationic lipid DOTMA. It is proposed that complexes form as a result of ionic interaction between the negatively charged DNA substitutes and the positive charge functional group of DOTMA. Cryo transmission electron microscopy has been used to analyse the cationic lipids combined with DOPE and varying quantities of three distinct cationic surfactants to ascertain the composite properties. The findings of the cryo TEM investigation recommend that DNA molecules may become caught between lamellae in collections of aggregated multilamellar forms due to an excess of charged lipids. The use of a surfactant does not affect the structure of the complexes.[11]

Immune liposome

The ability of liposomes to act as an immunological adjuvant to enhance the immune response is another potential use for them in medicine. Antigens that have been reconstituted into liposomal membranes or that have been incorporated into the aqueous core of the liposome will increase immune response by activating macrophages, producing antibodies, effectively inducing cytotoxic cells, and causing anticancer activity. Because liposomes are biodegradable, have little toxicity, are not immunogenic, and

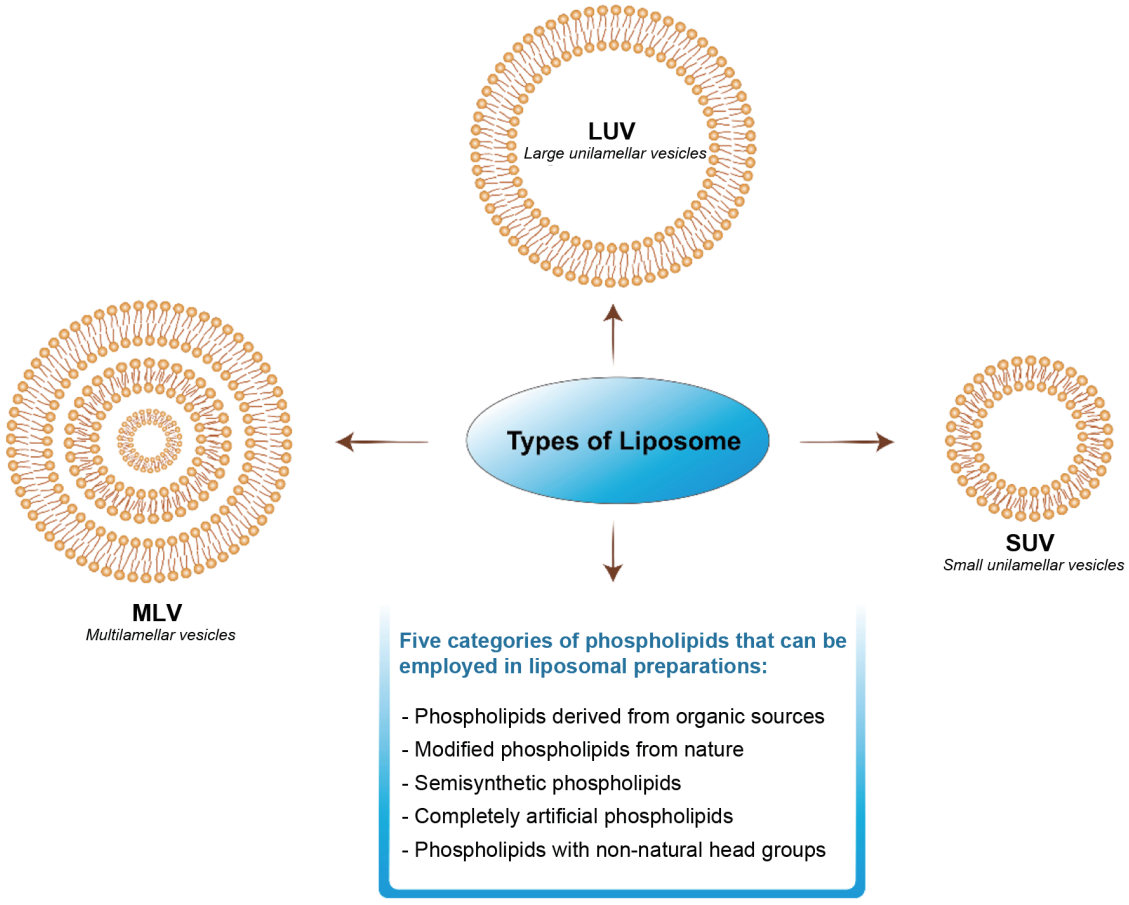

Fig. 1. Types of liposomes with categories of phospholipids.[5]

may target specific certain cells *in vivo*, they are advantageous as immunological adjuvants.[12] For improving the immunogenic potential of a specific antigen, such as glycolipids, proteins, and antigens to dangerous viruses, among others, liposomes are effective adjuvants. During *in vivo* tests antibody-sensitized liposomes, which have frequently shown highly positive outcomes in *in-vitro* investigations that frequently take place without macrophages, immunoglobulins, or components of the complement system, failed. Immune liposomes offer a practical alternative in immunoassays and diagnostic tests when taking into account a variety of other possible targeted applications, such as injection in various bodily cavities.[13]

Long stretching liposomes

The findings of the experiments demonstrate that liposomal disposition may be altered, including their intrahepatic uptake, especially within the mononuclear phagocytic system (MPS). Blood circulation times were lengthened, but the first observable benefit came when the bilayer was inserted with ganglioside GM1 or phosphatidylinositol at a concentration of 5–10% mol. To achieve the best results, both lipids were switched out for synthetic lipids that enclose polymers. Prolonged circulation was attained when the phospholipid and polyethylene glycol were covalently bonded.[14] It appears that a molecular component of 1,500–5,000 Da would be suitable, as the source of steric stabilisation is well documented but poorly defined. The existence of a steric shield is related to a decrease in the union and adsorption of blood products that marks unusual particles for subsequent macrophage uptake. The Alexander-de-Gennes hypothesis has recently been shown to be able to qualitatively assert the longevity of liposomes in living systems. The many liposome varieties are listed below in short form.[15] Different types of liposomes with their categories of phospholipids are described in Figure 1. Various advantages and disadvantages of liposomes are described in Table 1.

Table 1. Merits and Demerits of liposomes[5]

Merits	(1) Both negatively and positively charged compounds can bind with liposomes. (2) The DNA is somewhat protected from deteriorating processes by liposomes. (3) Liposomes are capable of transporting large amounts of DNA, maybe the size of a chromosome. (4) Targeting particular cells or tissues with liposomes is possible.
Demerits	(1) High production costs. (2) Leakage and fusion of drug- or molecule-encapsulated substances. (3) Phospholipid sometimes experiences reactions like oxidation and hydrolysis. (4) Short half-life. (5) Poor solubility.

Novel approaches for liposome production

Numerous techniques can be used to develop liposomes. As such, liposomal nomenclature depends on the preparation technique, structural parameters, or assigned special function.[16]

Liposome produced by hand shaking methods

Using a physical dispersion process and various lipid ratios, liposomes were produced. In this procedure, chloroform was used to dissolve the lipids. This conical flask has a flat bottom and is covered with the lipid solution in chloroform. The solution was then allowed to evaporate at room temperature without being disturbed. With phosphate buffer (pH 7.4) tilted to one side an aqueous medium containing medication was poured into the side of the test flask, and the test flask was gently brought back to an upright position. This process was used to hydrate the appearance of the lipid film. After the flask had fully inflated for two hours at 37°C, the fluid was allowed to flow silently over the lipid layer. Vesicles are obtained by swirling the flask's contents to produce a milky white suspension. Next, centrifugation was used for the formulations. A variety of liposome batches were created to find the best recipe. According to the procedure and lipid content for liposome manufacture stated above, all batches of liposomes have been produced.[16]

Liposome produced by sonication technique

The procedure most frequently used to prepare small, unilamellar vesicles (SUVs) is sonication. Multilamellar vesicle liposomes (MLVs) are sonicated in this situation using a passive atmosphere and either a probe-type sonicator or a bath-type sonicator. Sonication techniques, therefore, have two types, probe sonication and bath sonication. The liposome dispersion is promptly engulfed by the probe sonication technique. This approach has a very large energy input into the lipid dispersion. The vessels must be submerged in water or an ice bath since the coupling of energy at the tip causes localized heat. In the case of bath sonication, the dispersion directly utilises the tip, and the cylinder holding the liposomes is placed into a bath sonicator at a predetermined temperature, which is often a simpler procedure. In contrast to a probes unit, the substance being sonicated can be safeguarded in a sterile vessel or an inert environment.[17]

Liposome produced by micro-emulsification technique

The development of tiny vesicles from concentrated lipid solution requires the use of a micro-fluidizer. Large MLVs can be used to suspend the lipids before adding them to the fluid. The apparatus pushes fluid through a 5 mm screen at very high pressure. Next, it is forced along lengthy microchannels, causing two streams of fluid to collide at an extreme angle and speed. A pump and an interaction chamber can be used to recycle the collected fluid until spherical vesicles are produced.[7]

Liposome produced by a French pressure cell

The French pressure cell method comprises the extrusion of MLVs via a tiny aperture. An essential characteristic of this technique is that the protein does not appear to change dramatically as it does after sonication. The technique calls for delicate manipulation of unstable techniques. The approach provides several benefits over sonication. The end product is a liposome that is bigger than sonicated SUVs. The method's shortcomings include the difficulty in achieving the high temperature and the relatively modest working quantities.[7,17]

Liposome produced by ether injection method

A lipid solution is dissolved in ether, diethyl ether, or methanol and then gently injected into an aqueous solution of the substance to be encapsulated. Liposomes are created by successively removing the organic solvent under decreasing pressure. The major drawbacks of the method include heterogeneous populations and exposing the chemical that will be enclosed to organic solvents at extremely high temperatures.[18,19]

Liposome produced by ethanol injection method

The ethanol injection approach involves rapidly injecting an enormous amount of warmed distilled water or TRIS-HCl buffer with an ethanolic lipid solution. The hydrophilic/hydrophobic nature of the substance determines whether it will be incorporated into the liposomal vesicle. Compared to 5-fluorouracil, which migrates to the external aqueous phase, nimesulide is a lipid-soluble component that integrates better in liposomes. The inclusion of a non-harmful solvent in the ethanol injection technique is its principal benefit. The usefulness of such is limited by the potential for azeotrope production in water.[19,20]

Liposome produced by Freeze drying technique

By freeze-drying techniques water-soluble carrier materials and liposome-forming lipids that have been dissolved into tert-butyl alcohol/water, co-solvent solutions and isotropic monophasic solution cakes are formed. The freeze-dried product spontaneously creates a uniform dispersion of MLVs with the addition of water, which may subsequently be extruded to produce smaller MLVs. The size of the liposomes that were produced was below 200 nm, and the substances that were encapsulated affected the efficiency of encapsulation. Water-in-oil emulsions containing phospholipids were freeze-dried to produce lyophilates, which upon rehydration produced liposomes with an average size of less than 200 nm and encapsulation efficiency of more than 60% for three distinct medicines. The freeze-drying procedure additionally deals with the long-term liposome stability problems of thermolabile compounds, which are susceptible to heat drying. By removing water formation of liposomal products when they are frozen at incredibly low pressures while present with certain sugars (sucrose, trehalose), this technique prevents the leaking of encapsulated contents and the growth of liposome size following rehydration.[21]

Liposome produced by cross-flow filtration detergent depletion method

Production of detergent-mediated liposomes relies on the solubilization of lipids with the help of a suitable detergent, which creates mixed micelles. After that, the detergent is taken out, which causes the micelles to disintegrate and repack into lipid bilayers. The curved bilayers eventually contract and give way to unilamellar vesicles when the edge tension rises. However, the membranes will have a lot of detergent in them. Detergents that are membrane-bound might have detrimental effects on the bilayer. Therefore, a key step in detergent removal methods is the quick removal of detergent that has been membrane-bound. As such, a method that combines the benefits of established methods of detergent removal with quick and effective detergent removal can significantly cut down on preparing periods and different liposome lamellarity. Additionally, despite the technique being executed repeatedly and in sterile conditions, the process would be cost-effective and preferable. Liposomes with a predetermined size, homogeneity, and excellent stability may be produced using the cross-flow filtering method. When compared to other methods of detergent removal, many liposomes can be developed in a reduced timeframe. Additionally, using sterile filtered

combined micelles and autoclaved equipment is a starting point that allows for the creation of sterile products. To reduce the cost of production, the used filtrate can also be recycled.[22]

Liposomes application in the field of medicine

Liposomes can modify the temporal and spatial dispersal of drug molecules inside the body, thereby lowering dangerous side effects and enhancing therapeutic efficacy. The application of drug- or element-containing liposomes for diagnostic and therapeutic reasons, as well as their use as a shape, device, or reactant in basic research on cell interface recognition methods, in addition to the mechanism of action of particular materials, are examples of liposome applications in pharmacology and medicine. The way liposomes interact with cells and what happens to them *in vitro* after injection are key factors in determining the advantages and disadvantages of liposomal drug carriers. Studies of liposomes' interactions with cells *in vitro* and *in vivo* have revealed that either straightforward adsorption or subsequent endocytosis is the most common kind of contact. Cell membrane fusion occurs significantly less often. The fourth potential contact is the interchange of bilayer components with cell membrane components, such as cholesterol and lipid. The destiny of liposomes *in vivo* is likewise determined by such interactions. The body has a sophisticated defensive mechanism to protect itself. Larger things that enter the body induce thrombus development and eventually have their surfaces passivated by biomacromolecules, Conversely, immune system cells devour smaller particles along with germs, bacteria, and colloids. However, this immune response has considerably aided in the generation of biocompatible and unrecognizably different surfaces while also restricting the potential applications of microparticle drug delivery carriers to only those immune system cells. Liposomes are no different, even though they are made of natural components. The macrophages present in the liver, bone marrow, and spleen, swiftly remove liposomes from the bloodstream.[23]

Mode of action of liposomes

According to the previous discussion, liposomes differ from free drug particles in terms of bio-distribution and pharmacokinetics. Such differences may be employed in some circumstances to increase the medicinal potency of the encapsulated chemicals.[24] The advantages of liposomes containing active moieties can be delivered as aerosols, liquid solutions, or semi-solid forms like creams, and gels that among others can be categorized into seven groups:

Drugs that are amphiphilic and lipophilic have improved solubility

Hydrophilic medications, Some medications, such as the antiviral drug Acyclovir and the anticancer medication Doxorubicin, may be enclosed in the liposomal core at concentrations that are many times higher than their solubility in water. This is made feasible by the medication or gel structure precipitating within the liposome with the proper ingredients contained.[25]

Non-active targets for immune system cells

Examples include, antimonials, porphyrins, Amphotericin B, vaccinations, immunological modulators, and (immune) suppressors.[26]

Maintained a liposome-free system after local or systemic administration

Doxorubicin, cortisones, cytosine, arabinose, and biological proteins or peptides like vasopressin are some examples.[24]

Site-avoiding technique

The cardiotoxicity, nephrotoxicity, and neurotoxicity is reduced since liposomes do not degrade. Amphotericin B's decreased nephrotoxicity and Doxorubicin liposomes' diminished cardiotoxicity are characteristic test results.[26]

Targeting a location with precision

Target cells may occasionally interact with liposomes by surface-attached ligands, or liposomes may enter the targeted tissue through local anatomical characteristics including leaky, as well as, incorrectly constructed blood vessels, capillaries, and basal lamina.[27]

Better cellular uptake

Enhanced cell uptake of hydrophilic, electric compounds including plasmids, chelators, antibiotics, and genes.[28]

Improved tissue penetration, especially with dermally functioning liposomal dose forms

When administering medications locally (subcutaneously, intramuscularly, or intravenously), especially when they are toxic, potent, or have short blood circulation half-lives, liposome encapsulation is typically done with caution.[27]

Medical applications

Application in infections and parasitic diseases

Although following intravenous injection, liposomes are often broken down by phagocytic cells in the body, they are perfect delivery systems for drug molecules intended for these macrophages. Several parasite illnesses frequently harbour in the cells of the MPS. Such parasites are the best-known instances of a "Trojan horse-like" process. Liposomes are a perfect medication delivery method since they build up in the same infected cell population. Such formulations are moving away from the quickly growing and productive sector of liposome formulations in antifungal treatment, which frequently utilises the ionophore amphotericin B.[7] The dimensions of the drug molecule and its complex is commonly associated with toxicities, and liposome encapsulation undoubtedly inhibits drug accumulation in organs, significantly lowering toxicity. Antiviral and antibacterial therapies can employ similar strategies. Due to the interplay between substances with bilayers, as well as, the high density of aqueous systems, which frequently cause liposomes to stay on the top of the tube as a creamy layer, the production of liposomes containing antibiotics at relatively high drug-to-lipid ratios may be an intricated approach. A few different delivery strategies are also being considered, including topical and pulmonary delivery. There are various other ways to make use of the liposomes' inherent ability to target macrophages, such as macrophage activation and injection. Some common poisons induce a strong macrophage response. Since small molecules with immunogenic qualities (haptens) do not trigger an immune response when not connected to the bigger particles, such qualities may be replicated and strengthened by the use of liposomes. Generally, this is accomplished by administering alum or dead bacteria, but liposomes provide a viable alternative. Liposomes have been utilised in animal vaccines since 1988, while clinical studies for the malaria vaccine for humans are now underway.[28]

Anticancer therapy by liposomes

Early research primarily indicated reduced toxicity of the medicine

contained in a liposome; however, the majority of the time the drug molecules were not accessible, substantially impairing both effectiveness and reduced toxicity. Unfortunately, it was discovered that primary and secondary liver cancers shared this trait. It has been demonstrated that several liposome formulations of various anticancer drugs are less harmful than free medication as liposome encapsulation reduces the medication molecules' ability to go to specific tissues. Reduced harm outcomes include results for both acute and long-term toxicities. Applications in people typically showed decreased toxicity and improved administration acceptability, but research results negated optimism about their efficacy. Clinical investigations on a variety of formulations are at various stages and have shown contradictory findings.[29]

Liposomes changed surface characteristics in cancer treatment

New tactics, such as the liposomes with changed surface characteristics, which are used for the specific targeting of cancer and some other ill cells, are hampered by the immune system's rapid clearance of them from circulation. Due to their inability to extravasate, certain liposomes can serve as site-specific drug accumulations in some cases of topical delivery or the pulmonary delivery of liposome-based aerosols. Nevertheless, this rapid clearance presents a substantial obstacle for the majority of other applications.[29]

Role of sterically stabilized liposomes in the medical field

The results showed that there may be changes in liposome distribution; however, most of the changes occur within the MPS and intrahepatic absorption. Although primary and important breakthroughs were made, the extension of blood circulation times was achieved by inserting ganglioside GM1 or phosphatidylinositol into the bilayer. Both lipids were replaced with synthetic polymers that included lipids for the greatest outcomes. The usage of polyethylene glycol that was covalently bonded to the phospholipid resulted in the longest circulation durations.[3] The ideal molecular mass appears to be in the 1,500–5,000 Da range. It was theorised that steric barriers prevent blood components from adherence and adsorption, exposing the extraneous particles for further macrophage absorption. The source of steric stabilization is adequately reported, but a complete understanding is undocumented. Nonetheless, the Alexander-de-Gennes model of polymers at surfaces provides a qualitative explanation for the durability of liposomes in biological systems.[30]

Stealth liposome applications in the medical field

While such application makes use of tiny vesicles' capacity to exit blood circulation, earlier application calls for bigger liposomes (0.2mm). Sterically stabilised liposomes can be used as a localised drug depot or a long-acting repository for sustained drug release. A case of improved treatment efficiency of cytosine arabinose in the therapy, and the second is the impact of polypeptide vasopressin on the subcutaneous/intramuscular extended-release system. The duration of effect was for 30 days as opposed to several days for a free drug and about 7 days for the peptides included in common liposomes. The importance of such concepts is emphasised by the creation of genetically modified proteins and polypeptides that are hindered by fast blood absorption, degradation, and/or inactivation in the system. Stealth liposomes modified biodistribution as they accumulate at areas with permeable blood capillaries, such as tumours, as well as infections among others and could be useful for several further applications. Stealth liposomes are distributed across the skin instead of the liver, bone marrow, and spleen in the intact vasculature, eradicating the possibility of bringing dermatological and antiviral medications to such locations. While it has been demonstrated that administering empty stealth liposomes is well tolerated, using liposomes that are laden with powerful medications needs extensive toxicity and tolerance investigations.[31]

Stealth liposome applications in humans

Clinical experiments in humans confirmed good outcomes from anticancer drugs contained in stealth liposomes in preclinical tests. As a result of the drug being released from the liposomes and being encapsulated in circulating liposomes for about 7 days after injection, drug metabolites were estimated at cancer sites. Results showed that medicine was absorbed into tumours 4–10 times more readily as compared to a control group. In comparison with the distribution of free medication, the drug concentration in lesions is around ten times higher. A high effectiveness was achieved and in conclusion, it appears that the outcomes from treatment of a variety of malignancies will significantly improve with the use of stealth liposomes laden with anticancer medications. It was also proposed that stealth liposomes will display high efficiency in the management of infections, and inflammation among other maladies.[32] Different liposomal drug formulations in preclinical development and approved by clinical trials are described in Table 2.[33–45]

Liposomes as vaccination and gene therapy

Due to their unique features liposomes are being investigated for the treatment of different illnesses, as well as, for uses in immunisation. Examples include insulin, prostaglandins, antivirals, antibiotics, and steroids, among other uses. The transport of nucleic acids into cells is essential for recombinant DNA technologies, gene function research, and gene therapy. *In vivo* delivery is more difficult than *in vitro* delivery, which may rely on a variety of physical and chemical strategies. There are numerous colloidal particles in DNA-carrier systems: It has been demonstrated that cationic liposomes can bind to complexes of negatively charged DNA and that the protein encoded in the DNA plasmid will be produced by the target cells as a result of these complexes' capacity to transfect cells *in vitro*. Gene therapy, which heals cell disorders by tuning the genes, is favoured over *in vivo* delivery. It has been shown that systemic or localised administration of cationic lipid-based DNA complexes can transfect lung epithelial cells. The liposomes enhance the immunological response to the vaccine antigen by acting as an adjuvant and co-adjuvant transporter for viral glycolipids and glycoprotein.[46]

Liposomes in cancer therapy

The therapeutic efficacy of liposomal preparations might be increased, and the negative side effects related to chemotherapy could be decreased, by specifically targeting anticancer drugs against antigens produced on cancerous cells using certain ligands. Lung endothelial cells and solid tumour tissue were targeted simultaneously, a novel classification of lengthy immune liposome, or PEG immune liposome-attached antibodies, demonstrating a significantly better targeting potential than the conventional immune liposomes. Many exceedingly hazardous anticancer drugs depend on the targeted delivery of tumour tissue using pendant-type immune liposomes during chemotherapy. The addition of a fusogenic molecule that would impact liposomal proportions after their interaction with the targeted cells or their internalisation by endocytosis, is the main purpose of the pendant-type immunoliposome. In contrast to chemotherapy and radiation, photodynamic therapy (PDT) has minimal side effects that make it a standout cancer treatment. These findings imply that PDT can benefit from a

Table 2. Different liposomal drug formulations in preclinical development and approved by clinical trial

S.No.	Product approved by clinical trial	Route of administration	Active ingredient	Application	Name of the manufacturer	Reference
1.	Doxil	Intravenous	Doxorubicin-API	Breast carcinoma, Kaposi's sarcoma, and ovarian cancer	Sequus	33,34
2.	DaunoXome	Intravenous	Daunorubicin-API	Kaposi's sarcoma associated with AIDS	NeXstar Pharmaceuticals	35
3.	Depocyt	Spinal	Cytarabine/ Ara-C-API	Neoplastic meningitis	SkyPharma.	35
4.	Myocet	Intravenous	Doxorubicin-API	Cyclophosphamide-based combination treatment for metastatic breast carcinoma	Elan	34
5.	Mepact	Intravenous	Mifamurtide-API	osteosarcoma that is high-grade, treatable, and non-metastatic	Takeda	36
6.	Marqibo	Intravenous	Vincristine-API	lymphoblastic leukaemia	Talon	37
7.	Onivyde	Intravenous	Irinotecan-API	Fluorouracil & leucovorin combination treatment for metastatic pancreatic cancer	Merrimack	38
8.	Abelcet	Intravenous	Amphotericin B-API	severe invasive fungi infection	Sigma-Tau	39
9.	Ambisome	Intravenous	Amphotericin B-API	noxious, serious fungal infection	Sigma-Tau	39
10.	Amphotec	Intravenous	Amphotericin B-API	virulent fungi infections	Ben Venue	40
11.	Visudyne	Intravenous	Verteporphin-API	neovascularization of the choroids	Novartis	41
12.	DepoDur	Epidural	Morphine sulphate-API	Pain reduction	SkyPharma	42
13.	Exparel	Intravenous	Bupivacaine-API	Pain control	Pacira	43
14.	Epaxal	Intramuscular	Inactivated hepatitis A virus-strain RGSB	Hepatitis-A	Crucell, Berna	44
15.	Inflexal	Intramuscular	Hemaglutinine from influenza virus variants A and B that has been inactivated	Influenza	Crucell, Berna	45

long-circulating liposomal formulation of photosensitive drugs.[47]

Liposomes in antimicrobial therapy

Due to several characteristics that the mycobacteria and the host share, mycobacterial illnesses are treated differently from other bacterial diseases. Liposomal penicillin and neomycin were shown to be active alongside bacteria in a straightforward *in vitro* culture; however, chloramphenicol's antibacterial action was constrained by the liposome trap. When administered to rabbits intravenously, liposome encapsulation modifies the tissue distribution of gentamicin. Compared to free rifabutin rifabutin's activity against mycobacterium avium infection was significantly increased by liposomal inclusion. Rifampin's antitubercular activity was also noticeably boosted when it was loaded in egg phosphatidylcholine liposomes.[48]

Liposomes for respiratory diseases

In many different forms of respiratory illnesses, liposomes are frequently utilised. In comparison to regular aerosol, liposomal aerosol offers the following benefits: improved stability in the vast aqueous core, sustained release, reduced toxicity, and prevention of local irritation. AmBisome, fungisome, and mycoses are among the injectable liposome-based goods now available. The following factors must be satisfied for a lung-specific liposomal medication delivery system to be effective: lipid proportion, size, cost, drug-to-lipid ratio, and administration method.[49]

Liposomes for brain-targeted delivery

Liposomes can penetrate a human glioma and pass across the blood-brain barrier. Sulfatide and a monoclonal antibody were also used by researchers as sensory tools to improve the liposome's targeting ability. In rat brain tumours, large amounts of CHP-coated egg PC liposomes were also assembled. Rats implanted with the Fisher-344 strain and 9L-gliosarcoma received carotid injections of CHP-coated liposomes which were [14C]-DPPC-tagged. Thirty minutes after the liposome injection every tissue was removed.

Both with and without the CHP covering, the liposome's tissue distribution was studied. When compared to the control liposome, the biodistribution of the CHP-coated liposome is augmented by 4.5-fold in the tumour and 2.1-fold increment in the ipsilateral brain, while there is a 4-fold drop in the spleen. Using liposomes that had an anticancer medication inserted into them, the lifetime of 9L-glioma-implanted rats was studied. The average survival duration for the group receiving CHP-coated liposomes was 35.3 days. This was statistically significant in comparison to the untreated group.[50]

Targeted long-circulating liposomes

Such liposomes endure in circulation for a prolonged duration to attain appropriate concentration which facilitates binding. Liposome formation was a crucial step in such binding, which after the ligands' coupling at the liposomal surface, continued to circulate for a long time. The liposomes' clearance rate was only slightly decreased by methods to prolong their circulation duration, such as shrinking their size or adding cholesterol and/or lipids with high phase transitions. Placing a hydrophilic molecule, such as GMi, phosphatidylinositol, poly(acrylamide), poly(vinylpyrrolidone), or poly (ethylene glycol), at the liposomal surface is a method of preventing lipolysis (methyl or ethyl oxazoline), was more successful. Poly (either methyl or ethyl oxazoline). It has been demonstrated that both GMi and PEG have been effective in extending the circulation period of liposomes loaded with therapeutic agents, even if not all of the aforementioned polymers have been examined in this respect.[51]

Liposome use in anti-bacterial medication

The behaviour of liposomes made of PEG-DSPE has been investigated: PHEPC was examined in a pneumonic rat model. Such liposomes have approximately a 20-hour half-life in blood circulation. Hepatosplenic absorption of the liposomes was not particularly effective but such liposomes are gradually directed toward the damaged lung tissue after being given intravenously. In this experimental pneumonia model, the effectiveness of gentamicin or ceftazidime contained in liposomes was examined. The liposome-encapsulated antibiotic's therapeutic efficacy was found to be superior to the free antibiotic at a single-dose treatment schedule that started 24 hours after bacterial inoculation in aspects of both enhanced elimination of bacteria in the infected lung tissue and supporting the survival of infected rats. In general, antibiotic-containing liposomes maintain good stability over prolonged blood circulation.[52]

Liposome use in antifungal medication

At therapeutically effective dosages, AmBisome® exhibits extended blood residence time; in people, the elimination half-life is approximately 32 hours. AmBisome® is often used at significantly greater dosages, and such increased levels have an improved antifungal impact. The release of AMB from AmBisome® close to fungus or direct interaction between AmBisome® and the fungal cell at the site of infection affects the activity of AmBisome® in severely infected organisms. In our lab, sterically stabilised AMB-containing liposomes were created to improve the circulation of AMB liposomes without being restricted by a high lipid dose.[52]

Sustained activity of liposomes

Currently, the liposomal drug delivery system is very popular for its sustained action and release of drugs without showing any toxicity inside the body. Such sustained activity of liposomes is only because of the encapsulated nature of the liposome, termed Liposomal Encapsulation Technology (LET). As such, the most recent delivery method employed by medical researchers to transport medications that operate as curative promoters to the guaranteed bodily parts is liposomal encapsulation technology (LET). Such a delivery system concept focused on getting critical substances into the body. LET is a technique for producing liposomes, which are sub-microscopic foams that can contain a variety of substances. Sub-microscopic foams create a barrier surrounding their contents that is impermeable to free radicals, digestive enzymes from the mouth and stomach, alkaline solutions, digestive juices, bile salts, as well as, intestinal flora produced by humans. Thus, the liposomes' contents are shielded from oxidation and destruction until the liposome's contents are transported to the precise target gland, organ, or system where they will be used as their protective phospholipid shell or barrier is unharmed. Such crosslinking technique for liposomes is considered for the site-specific and sustained release of medication. Here, the authors discuss some marketed formulations of liposomes that have sustained activity.[53] Table 3 describes the different liposomal products that are already available in the market.

Future perspectives

Liposomes have shown great potential in various medical applications and their prospects continue to be promising. Liposomes may be further optimized to enhance drug stability, improve targeting to specific tissues or cells, and control drug release rates. Optimization could lead to more effective and personalized treatments for various diseases, including cancer, infectious diseases, and genetic disorders. Researchers may also be involved in developing liposomes with enhanced transfection efficiency, increased stability, and improved targeting capabilities, allowing for precise gene delivery, as well as, potential treatment of genetic diseases. Novel liposomal formulations may involve designing liposomal vaccines that can induce stronger and longer-lasting immune responses against infectious diseases, cancers, and emerging pathogens. Liposomes can also be utilized for targeted imaging of specific tissues or cells which allows diagnostic tools to be more sensitive, specific, and multifunctional, enabling earlier detection, accurate diagnosis, as well as, monitoring of various diseases. Multifunctional liposomes can simultaneously deliver therapeutic agents and imaging agents, allowing for personalized medicine approaches. Future developments may focus on optimizing theranostic liposomes to achieve synergistic effects, real-time monitoring of treatment responses, and individualized treatment regimens. Future advancements may involve developing liposomal formulations that can improve stem cell therapy outcomes, enhance tissue engineering approaches, and facilitate the regeneration of damaged organs or tissues. Liposomes hold significant promise in these areas, further research and development are required to overcome challenges such as stability, scalability, and regulatory approval. However, the continued advancements in liposome technology and our understanding of their interactions with biological systems suggest a bright future for their medical application.

Conclusion

The development of liposomes as novel carriers has been a consequence of collaborative efforts in investigations throughout the last 2 to 3 decades, enlisting different fields of science, such as chemical, colloidal, physical, and biological among others. The established

Table 3. Marketed liposomal product[53]

Sl. No.	Marketed product	Active ingredient	Dosage form	Route of administration
1.	Doxil Caelyx	Doxorubicin hydrochloride	Liposomal Suspension	Intravenous
2.	DaunoXome	Daunorubicin	Liposomal Suspension	Intravenous
3.	AmBisome	Amphotericin B	Lyophilized	Intravenous
4.	DepoCyte	Cytarabine	Liposomal Suspension	Intrathecal
5.	Myocet	DOX-HCL	Liposomal Suspension	Intravenous
6.	Visudyne	Verteporfin	Lyophilized	Intravenous
7.	DepoDur	Morphine	Liposomal Suspension	Epidural
8.	Exparel	Bupivacaine	Liposomal Suspension	Intravenous
9.	Marqibo	Vincristine sulphate,	Liposomal Suspension	Intravenous
10.	Onivyde	Irinotecan hydrochloride trihydrate	Liposomal Suspension	Intravenous
11.	Vyxeos	Daunorubicin, cytarabine	Lyophilized	Intravenous
12.	Shingrix	Recombinant varicella virus-zoster glycoprotein E	Liposomal Suspension	Intramuscular
13.	Arikayce	Amikacin sulfate	Liposomal Suspension	Oral Inhalation

theoretical and investigational foundations hold promise for innovations and products. The uses, and assumptions, in particular, that will be effective remains uncertain. However, based on the products in the current market, the authors conclude that liposomes have unquestionably cemented their place in contemporary technology. The large-scale development of topically administered liposomal products, especially those made from lipid mixes with a structure comparable to that of the stratum corneum, is one such successful technology. The distribution method for the treatment of skin conditions would also be advantageous. With the advancement of other technologies, the study of liposomes will grow more complex, especially with an adequate platform for the creation of more favourable products, such as in the fields of public health and diagnostics.

Acknowledgments

None.

Funding

No funding has been received for this manuscript.

Conflict of interest

None.

Author contributions

Planning, drafting and/or critical revision of the article (BB and BGP), proofreading (BB, BGP, AD, HP), and supervision (BGP). All authors approved the final manuscript.

References

[1] Salimi A. Liposomes as a Novel Drug Delivery System: Fundamental and Pharmaceutical Application. Asian Journal of Pharmaceutics 2018;12:1. doi:10.22377/ajp.v12i01.2037.

[2] Nakhaei P, Margiana R, Bokov DO, Abdelbasset WK, Jadidi Kouhbanani MA, Varma RS, *et al*. Liposomes: Structure, Biomedical Applications, and Stability Parameters with Emphasis on Cholesterol. Front Bioeng Biotechnol 2021;9:705886. doi:10.3389/fbioe.2021.705886, PMID:34568298.

[3] Lasic DD, Frederik PM, Stuart MC, Barenholz Y, McIntosh TJ. Gelation of liposome interior. A novel method for drug encapsulation. FEBS Lett 1992;312(2-3):255–258. doi:10.1016/0014-5793(92)80947-f, PMID:1426260.

[4] Litzinger DC, Huang L. Phosphatidylethanolamine liposomes: drug delivery, gene transfer and immunodiagnostic applications. Biochim Biophys Acta 1992;1113(2):201–227. doi:10.1016/0304-4157(92)90039-d, PMID:1510997.

[5] Daraee H, Etemadi A, Kouhi M, Alimirzalu S, Akbarzadeh A. Application of liposomes in medicine and drug delivery. Artif Cells Nanomed Biotechnol 2016;44(1):381–391. doi:10.3109/21691401.2014.953633, PMID:25222036.

[6] Wagner A, Platzgummer M, Kreismayr G, Quendler H, Stiegler G, Ferko B, *et al*. GMP production of liposomes—a new industrial approach. J Liposome Res 2006;16(3):311–319. doi:10.1080/08982100600851086, PMID:16952884.

[7] Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, *et al*. Liposome: classification, preparation, and applications. Nanoscale Res Lett 2013;8(1):102. doi:10.1186/1556-276X-8-102, PMID:23432972.

[8] Aramaki K, Watanabe Y, Takahashi J, Tsuji Y, Ogata A, Konno Y. Charge boosting effect of cholesterol on cationic liposomes. Colloid Surface A 2016;506:732–738. doi:10.1016/j.colsurfa.2016.07.040.

[9] Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int J Nanomedicine 2006;1(3):297–315. PMID:17717971.

[10] Wang CY, Huang L. pH-sensitive immunoliposomes mediate target-cell-specific delivery and controlled expression of a foreign gene in mouse. Proc Natl Acad Sci U S A 1987;84(22):7851–7855. doi:10.1073/pnas.84.22.7851, PMID:2446313.

[11] Gustafsson J, Arvidson G, Karlsson G, Almgren M. Complexes between cationic liposomes and DNA visualized by cryo-TEM. Biochim Biophys Acta 1995;1235(2):305–312. doi:10.1016/0005-2736(95)80018-b, PMID:7756339.

[12] LeGrue SJ. Carrier and adjuvant properties of liposome-borne tumor-specific antigens. Cancer Immunol Immunother 1984;17(2):135–141. doi:10.1007/BF00200050, PMID:6565518.

[13] Alving CR. Liposomes as carriers of antigens and adjuvants. J Immunol Methods 1991;140(1):1–13. doi:10.1016/0022-1759(91)90120-5, PMID:1712030.

[14] Allen TM, Chonn A. Large unilamellar liposomes with low uptake into the reticuloendothelial system. FEBS Lett 1987;223(1):42–46. doi:10.1016/0014-5793(87)80506-9, PMID:3666140.
[15] De Gennes PG. Polymers at an interface; a simplified view. Advances in Colloids and Interface Science 1987;27(1):189–209.
[16] Pradhan B, Kumar N, Saha S, Roy A. Liposome: method of preparation, advantages, evaluation and its application. Journal of Applied Pharmaceutical Research 2015;3(3):1–8.
[17] Riaz M. Liposomes preparation methods. Pak J Pharm Sci 1996; 9(1):65–77. PMID:16414777.
[18] Kumar A, Badde S, Kamble R, Pokharkar VB. Development and characterization of liposomal drug delivery system for nimesulide. Int J Pharm Pharm Sci 2010;2(4):87–89.
[19] Marie M, Habeeb AD. Preparation and evaluation of salbutamol liposomal suspension using chloroform film method. Mustansiriya Medical Journal 2012;11(2):39–44.
[20] Yamauchi M, Tsutsumi K, Abe M, Uosaki Y, Nakakura M, Aoki N. Release of drugs from liposomes varies with particle size. Biol Pharm Bull 2007;30(5):963–966. doi:10.1248/bpb.30.963, PMID:17473443.
[21] Patil YP, Jadhav S. Novel methods for liposome preparation. Chem Phys Lipids 2014;177:8–18. doi:10.1016/j.chemphyslip.2013.10.011, PMID:24220497.
[22] Huang Z, Li X, Zhang T, Song Y, She Z, Li J, *et al*. Progress involving new techniques for liposome preparation. Asian J Pharm Sci 2014;9(4):176–182. doi:10.1016/j.ajps.2014.06.001.
[23] Bangham AD. Properties and uses of lipid vesicles: an overview. Ann N Y Acad Sci 1978;308:2–7. doi:10.1111/j.1749-6632.1978.tb22010.x, PMID:279288.
[24] Taylor TM, Davidson PM, Bruce BD, Weiss J. Liposomal nanocapsules in food science and agriculture. Crit Rev Food Sci Nutr 2005;45(7-8):587–605. doi:10.1080/10408390591001135, PMID:16371329.
[25] Samad A, Sultana Y, Aqil M. Liposomal drug delivery systems: an update review. Curr Drug Deliv 2007;4(4):297–305. doi:10.2174/156720107782151269, PMID:17979650.
[26] Johnston MJ, Semple SC, Klimuk SK, Ansell S, Maurer N, Cullis PR. Characterization of the drug retention and pharmacokinetic properties of liposomal nanoparticles containing dihydrosphingomyelin. Biochim Biophys Acta 2007;1768(5):1121–1127. doi:10.1016/j.bbamem.2007.01.019, PMID:17321495.
[27] Perrie Y, Frederik PM, Gregoriadis G. Liposome-mediated DNA vaccination: the effect of vesicle composition. Vaccine 2001;19(23-24):3301–3310. doi:10.1016/s0264-410x(00)00432-1, PMID:11312029.
[28] Bakker-Woudenberg IA, Storm G, Woodle MC. Liposomes in the treatment of infections. J Drug Target 1994;2(5):363–371. doi:10.3109/10611869408996811, PMID:7704480.
[29] Weiner N, Martin F, Riaz M. Liposomes as a drug delivery system. Drug Dev Ind Pharm 1989;15(10):1523–1554. doi:10.3109/03639048909052502.
[30] Patel N, Panda S. Liposome drug delivery system: a critic review. Journal of pharmaceutical Science and Bioscientific Research 2012;2(4):169–175.
[31] Agarwal R, Katare OP, Vyas SP. Preparation and in vitro evaluation of liposomal/niosomal delivery systems for antipsoriatic drug dithranol. Int J Pharm 2001;228(1-2):43–52. doi:10.1016/s0378-5173(01)00810-9, PMID:11576767.
[32] Ainbinder D, Paolino D, Fresta M, Touitou E. Drug delivery applications with ethosomes. J Biomed Nanotechnol 2010;6(5):558–568. doi:10.1166/jbn.2010.1152, PMID:21329048.
[33] Slingerland M, Guchelaar HJ, Gelderblom H. Liposomal drug formulations in cancer therapy: 15 years along the road. Drug Discov Today 2012;17(3-4):160–166. doi:10.1016/j.drudis.2011.09.015, PMID:21983329.
[34] Leonard RC, Williams S, Tulpule A, Levine AM, Oliveros S. Improving the therapeutic index of anthracycline chemotherapy: focus on liposomal doxorubicin (Myocet). Breast 2009;18(4):218–224. doi:10.1016/j.breast.2009.05.004, PMID:19656681.
[35] Gill PS, Espina BM, Muggia F, Cabriales S, Tulpule A, Esplin JA, *et al*. Phase I/II clinical and pharmacokinetic evaluation of liposomal daunorubicin. J Clin Oncol 1995;13(4):996–1003. doi:10.1200/JCO.1995.13.4.996, PMID:7707129.
[36] Meyers PA, Schwartz CL, Krailo MD, Healey JH, Bernstein ML, Betcher D, *et al*. Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival—a report from the Children's Oncology Group. J Clin Oncol 2008;26(4):633–638. doi:10.1200/JCO.2008.14.0095, PMID:18235123.
[37] Webb MS, Harasym TO, Masin D, Bally MB, Mayer LD. Sphingomyelin-cholesterol liposomes significantly enhance the pharmacokinetic and therapeutic properties of vincristine in murine and human tumour models. Br J Cancer 1995;72(4):896–904. doi:10.1038/bjc.1995.430, PMID:7547237.
[38] Drummond DC, Noble CO, Guo Z, Hong K, Park JW, Kirpotin DB. Development of a highly active nanoliposomal irinotecan using a novel intraliposomal stabilization strategy. Cancer Res 2006;66(6):3271–3277. doi:10.1158/0008-5472.CAN-05-4007, PMID:16540680.
[39] Janoff AS, Boni LT, Popescu MC, Minchey SR, Cullis PR, Madden TD, *et al*. Unusual lipid structures selectively reduce the toxicity of amphotericin B. Proc Natl Acad Sci U S A 1988;85(16):6122–6126. doi:10.1073/pnas.85.16.6122, PMID:3413081.
[40] Janoff AS, Perkins WR, Saletan SL, Swenson CE. Amphotericin B Lipid Complex (Ablc™): A Molecular Rationale for the Attenuation of Amphotericin B Related Toxicities. Journal of Liposome Research 1993;3(3):451–471. doi:10.3109/08982109309150730.
[41] Woodle MC, Storm G. Long circulating liposomes: old drugs, new therapeutics 2013;Springer.
[42] Alam M, Hartrick CT. Extended-release epidural morphine (DepoDur): an old drug with a new profile. Pain Pract 2005;5(4):349–353. doi:10.1111/j.1533-2500.2005.00048.x, PMID:17177768.
[43] Richard BM, Rickert DE, Newton PE, Ott LR, Haan D, Brubaker AN, *et al*. Safety Evaluation of EXPAREL (DepoFoam Bupivacaine) Administered by Repeated Subcutaneous Injection in Rabbits and Dogs: Species Comparison. J Drug Deliv 2011;2011:467429. doi:10.1155/2011/467429, PMID:22013534.
[44] Mayorga Pérez O, Herzog C, Zellmeyer M, Loáisiga A, Frösner G, Egger M. Efficacy of virosome hepatitis A vaccine in young children in Nicaragua: randomized placebo-controlled trial. J Infect Dis 2003;188(5):671–677. doi:10.1086/377309, PMID:12934183.
[45] Glück R, Mischler R, Finkel B, Que JU, Scarpa B, Cryz SJ Jr. Immunogenicity of new virosome influenza vaccine in elderly people. Lancet 1994;344(8916):160–163. doi:10.1016/s0140-6736(94)92758-8, PMID:7912766.
[46] Anderson M, Omri A. The effect of different lipid components on the in vitro stability and release kinetics of liposome formulations. Drug Deliv 2004;11(1):33–39. doi:10.1080/10717540490265243, PMID:15168789.
[47] Aoki H, Mizuno M, Natsume A, Tsugawa T, Tsujimura K, Takahashi T, *et al*. Dendritic cells pulsed with tumor extract-cationic liposome complex increase the induction of cytotoxic T lymphocytes in mouse brain tumor. Cancer Immunol Immunother 2001;50(9):463–468. doi:10.1007/s002620100220, PMID:11761440.
[48] Awasthi VD, Garcia D, Goins BA, Phillips WT. Circulation and biodistribution profiles of long-circulating PEG-liposomes of various sizes in rabbits. Int J Pharm 2003;253(1-2):121–132. doi:10.1016/s0378-5173(02)00703-2, PMID:12593943.
[49] Fendler JH, Romero A. Liposomes as drug carriers. Life Sci 1977;20(7):1109–1120. doi:10.1016/0024-3205(77)90481-7, PMID:403379.
[50] Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Matthay K, Huang SK, *et al*. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc Natl Acad Sci U S A 1991;88(24):11460–11464. doi:10.1073/pnas.88.24.11460, PMID:1763060.
[51] Ashok B, Arleth L, Hjelm RP, Rubinstein I, Onyüksel H. In vitro characterization of PEGylated phospholipid micelles for improved drug solubilization: effects of PEG chain length and PC incorporation. J Pharm Sci 2004;93(10):2476–2487. doi:10.1002/jps.20150, PMID:15349957.
[52] Senior J, Delgado C, Fisher D, Tilcock C, Gregoriadis G. Influence of surface hydrophilicity of liposomes on their interaction with plasma protein and clearance from the circulation: studies with poly (ethylene glycol)-coated vesicles. Biochimica ET Biophysica Acta-Biomembranes 1991;1062(1):77–82. doi:10.1016/0005-2736(91)90337-8.
[53] Liu P, Chen G, Zhang J. A Review of Liposomes as a Drug Delivery System: Current Status of Approved Products, Regulatory Environments, and Future Perspectives. Molecules 2022;27(4):1372. doi:10.3390/molecules27041372, PMID:35209162.