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A Comprehensive Review on Preparation Application and New Generation of Liposomes

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A B S T R A C T

Liposomes, sphere- shaped vesicles conforming of one or further phospholipid bilayers, were first described in themid-60s. moment, they're a veritably useful reduplication, reagent, and tool in colorful scientific disciplines, including mathematics and theoretical drugs, biophysics, chemistry, colloid wisdom, biochemistry, and biology. Since also, liposomes have made their way to the request. Among several talented new medicine delivery systems, liposomes characterize an advanced technology to deliver active motes to the point of action, and at present, several phrasings are in clinical use. exploration on liposome technology has progressed from conventional vesicles to' alternate- generation liposomes', in which long- circulating liposomes are attained by modulating the lipid composition, size, and charge of the vesicle. Liposomes with modified shells have also been developed using several motes, similar as glycolipids or sialic acid. This paper summarizes simply scalable ways and focuses on strengths, independently, limitations in respect to artificial connection and nonsupervisory conditions concerning liposomal medicine phrasings grounded on FDA and EMEA documents.

Keywords Liposome, Phospholipids, ULV, MLV, Drug Delivery System

Introduction

Liposomes were discovered by Alec D Bangham in the 1960s at the Babraham Institute, University of Cambridge, and correspond of single or multiple concentric lipid bilayers recapitulating an waterless cube (2). The first phrasings were composed solely of natural lipids; at present they can include natural and/ or synthetic lipids and surfactants. They've the capability of enmeshing both lipophilic and hydrophilic agents, in the lipid membrane and in the waterless core, independently. The size of these nearly globular lipid vesicles can range from a many nanometers to several micrometers. still, liposomes applied to medical use range between 50 and 450 nm (3)

Liposomes feel to be an nearly ideal medicine- carrier system, since their morphology is analogous to that of cellular membranes and because of their capability to incorporate colorful substances. thus, for the last 50 times liposomes have been extensively delved and they continue to be the subject of violent exploration. They're valued for their natural and technological advantages as optimal delivery systems for biologically active substances, both in vitro and in vivo, and are considered to be the most successful medicine- carrier system known to date(4). During the two last decades, notable progress has been made, and several biomedical operations of liposomes are moreover in clinical trials or are about to be put on the request, while others have formerly been approved for public use(5).

Raw Material of Liposome

Lipids are amphipatic motes with water-friendly and water- abhorring corridor(figure 2). Liposomes are comported of single or multiple lipid bilayers formed by hydrophilic and hydrophobic relations with the waterless phase. The hydrophobic corridor(tails) of liposomes are repelled by water motes performing in liposome tone assembly(6). In addition, Phosphatidylcholine(PC) and Dipalmitoyl PC can be used for liposome generation, independently. Two important advantages of liposomes, in medicine delivery of living organisms, are biocompatibility and biodegradability, which are due to lipid characteristics(7). Different types of lipids and amphiphiles can act as liposomes like phosphotidylcholine, phosphotidylserine, phosphotidylethanolamine. likewise, polymers can be used for the conflation of polymerosomes as new medicine/ gene carriers.

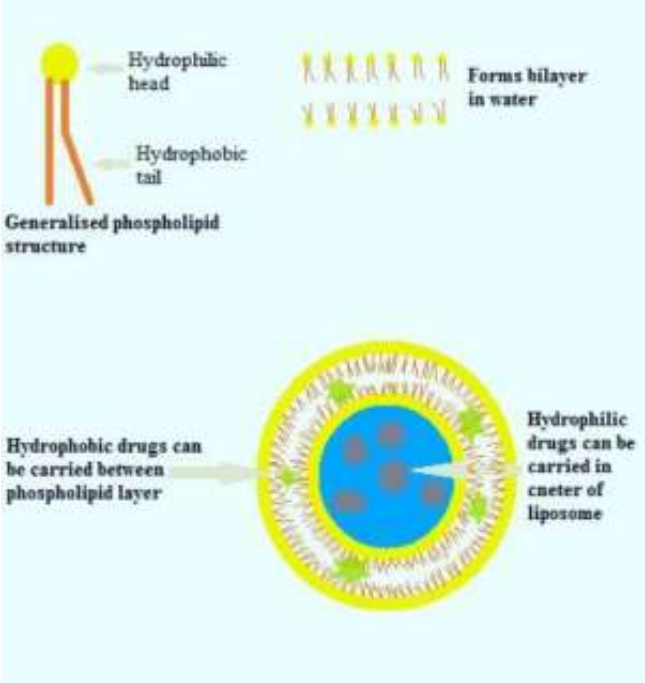


Figure 2: Schematic Drawing of Liposome Structure and Its Compartments

Composition of liposomes

The major components involved in liposomal preparations are *phospholipids* and *cholesterol*.

Phospholipids:

Glycerol containing phospholipids are most commonly used for liposome formulations. They represent more than 50% of the weight of lipid present in the biological membranes. Saturated fatty acids are used in order to produce stable liposomes. Most abundant glycerol phospholipids in plants and animals are phosphatidyl choline (PC) also known as lecithin and phosphatidyl ethanolamine (PE), sometimes referred to as cephalin [35]. Phospholipids used for liposomal preparation can be of following varieties [34]. A comprehensive list of commonly used phospholipids and their phase transition temperatures (Tc) are summarized in Table 1.

- Natural phospholipids
- Synthetic phospholipids
- Semi-synthetic phospholipids
- Modified natural phospholipids
- Phospholipids having non-natural phospholipids

Examples:

Phosphatidyl choline, Phosphatidyl ethanolamine, Phosphatidyl inositol,

Phosphatidyl glycerol, Phosphatidyl serine.

Table 1. List of commonly used phospholipids and their phase transition temperatures (Tc)

Phospolipids	**Tc (°C)**
Soybean phosphatidylcholine (SPC)	-20 to-30
Hydrogenated soybean phosphatidylcholine (HSPC)	52
Egg sphingomyelin (ESM)	40
Egg phosphatidylcholine (EPC)	-5 to-15
Dimyristoyl phosphatidylcholine (DMPC)	23
Dipalmitoyl phosphatidylcholine (DPPC)	41
Dioleoyl phosphatidylcholine (DOPC)	-22
Distearoyl phosphatidylcholine (DSPC)	55
Dimyristoyl phosphatidylglycerol (DMPG)	23

Dipalmitoyl phosphatidylglycerol (DPPG)	41
Dioleoyl phosphatidylglycerol (DOPG)	-18
Distearoyl phosphatidylglycerol (DSPG)	55
Dimyristoyl phosphatidylethanolamine (DMPE)	50
Dipalmitoyl phosphatidylethanolamine (DPPE)	60
Dioleoyl phosphatidylethanolamine (DOPE)	-16
Dimyristoyl phosphatidylserine (DMPS)	38
Dipalmitoyl phosphatidylserine (DPPS)	51
Dioleoyl phosphatidylserine (DOPS)	-10

Cholesterol:

Cholesterol is added to improve the characteristics of lipid bilayers. The ratio of cholesterol to phosphotidylcholine can be in the ratio of 1:1 or 1:2. It improves the membrane fluidity, bilayer stability and reduces the permeability of water-soluble molecules through the membrane [36]. Preparation of liposomes [37]. There are two mechanisms by which vesicles formation occurs as follows,

A) The budding theory

B) The bilayer phospholipids theory

A) Budding theory:

This theory suggests that, liposomes are formed by the stress induced hydration of phospholipids. That's why, the phospholipids get organized into lamellar arrays which results into budding of lipid bilayers and finally leads to downsizing.

B) The bilayer phospholipids theory:

According to this theory, The formation of liposomes by hydration of thin lipid films. During the process of agitation, the hydrated lipid films/ sheets gets separated and self-close to form large, multilamellar vesicles.

The Physicochemistry of Liposomes

The acceptability of liposomes as a carrier system for medicines rigorously depends on the physicochemical parcels of their membranes, on the nature of their factors, on their size, face charge, and lipid association(8). Liposomes are substantially composed of phospholipids, amphiphilic motes that have a hydrophilic head and two apolar hydrophobic chains. When phospholipids are dispersed in waterless results, due to their amphipathic nature they've a strong tendency to form membranes(9). On the one hand, their polar heads prefer to interact with the waterless terrain; on the other, their long apolar aliphatic chains promote commerce with one another. In waterless result, these binary parcels favor the conformation of two lipid layers. The hydrophobic chains of each subcaste face each other and constitute a lipophilic inner cube that acts as a permeability hedge, both inward and outward. Hydrophobic relations are behind the conformation of these lipid bilayers, and van der Waals forces keep the long hydrocarbon tails together, therefore strengthening this armature. Incipiently, hydrogen bonds and polar relations between the water motes of the waterless terrain and the polar heads of lipids stabilize this association. The final association of lipids depends on their nature, attention, temperature, and geometric form(10). still, they can be reprised inside these membranes, If ions or motes are present during the expression process.

Liposomes can be classified on the base of the medication system(rear- phase evaporation vesicles or vesicle extruded fashion), size(small, intermediate, or large), and lamellarity(uni-oligo, and multilamellar vesicles). The conformation of unilamellar vesicles(ULVs) or multilamellar vesicles(MLVs) depends on the conflation styles and postformation processing used for their medication(relate to the " styles for the medication of liposomes " section for further details). Since ULVs(one lipid bilayer, 50 – 250 nm) enclose a large waterless core, they're immaculately suited for the encapsulation of hydrophilic medicines. On the other hand, MLVs(two or further concentric lipid bilayers organized like an onion- skin, 1 – 5 μm) preferentially entrap lipid-answerable medicines(11). In addition to the capability to entrap medicines with different solubility characteristics, it has been hypothecated that ULVs and MLVs have different release kinetics. In general, MLVs are formed more fluently at larger hydrodynamic compasses, and therefore have lesser entrapped volume than ULVs. As a result, unilamellar liposomes with a hydrodynamic periphery of 130 nm parade a important faster release rate than MLVs with two to three lamellar bilayers and a hydrodynamic periphery of 250 nm(,13). The difference in the release rate is due overall to the number of phospholipid bilayer that it have to cross before being released.

Pharmacokinetics of liposome

1. Absorption

The fraction of therapeutically active drug that reaches the systemic circulation from any route of administration is defined as its bioavailability. A major factor that determines the bioavailability of a drug is its extent of absorption from the site of administration into the general circulation. In terms of a liposome-based drug delivery system, the fraction of drug and/or carrier that reaches the systemic circulation depends on the liposome composition, the characteristics of the drug, the route of administration and the physiology of the absorption site. To date, most applications of SL involve parenteral

(intravenous) administration. However, a pharmacokinetic analysis has been made for liposomes (SL and CL) following administration by both the subcutaneous (s.c.) and intraperitoneal (i.p.) routes of administration.

2. Distribution

After the administration of free drug into the systemic circulation, or following its absorption from another site, the drug distributes in vivo depending on its hydrophilicity and molecular size. Many small molecules and ions distribute rapidly in plasma by diffusional processes, pass through capillaries and eventually through cell membranes. Large hydrophilic macromolecules (50 kDa or more) may require energy-dependent transport mechanisms in order to leave the circulation and/or enter cells. Amphipathic molecules, even fairly large ones, are able to easily move in both directions across biological membranes, while strongly hydrophobic molecules are retained by lipid membranes. All drug carriers, including liposomes which have apparent molecular weights in the millions, are very large compared with the size of clinical therapeutic agents. Because of their large size, the distribution of liposomes and other drug carriers is highly restricted compared to the distribution of their associated drugs. Therefore, encapsulation into liposomes may markedly influence the biodistribution of drugs. The distribution of liposome-associated drugs is dependent on both the rate of release of drug from the carrier and the restricted accessibility of the carrier to tissues and interstitial fluids and the release of the drug therein.

3. Clearance of Liposomes:

A primary property of liposomes that makes them valuable as drug delivery systems is their ability to change the pharmacokinetics of their associated drugs [14,15]. Relative to the same drugs in aqueous solution (i.e. 'free' drug), significant changes in absorption, biodistribution and clearance of liposome-associated drugs are apparent, resulting in dramatic effects on both the efficacy and toxicity of the entrapped compound. After intravenous administration, the liposomes, together with their associated drug, will circulate in the blood with half-lives determined by the liposome size and composition. For example, the smaller liposomes have slower clearance rates than the larger conventional liposomes [15]. It can be seen that, relative to their respective free drugs, pegylated liposomal doxorubicin has the lowest clearance rate, followed by liposomal daunorubicin (DaunoXome®) and, finally, TLCD99 doxorubicin [16,17,18].

4. Long-Circulating Liposomes:

The clearance rates of liposomes are most substantially decreased (i.e, the circulation half-lives increased) by grafting hydrophilic polymers such as polyethylene glycol (PEG) onto the liposome surface (e.g. Stealth® liposomes) [19,20]. This strategy not only increases the residence time of liposomes in the vasculature but also changes the pharmacokinetics from dose dependent (saturable) to the relatively dose-independent pharmacokinetics within the clinical dose range.[21,22].

5. Elimination of Liposomes:

The principle site of clearance of liposomes and their associated drugs from the blood is the mononuclear phagocyte system (MPS), comprised principally of Kupffer cells in the liver and fixed macrophages in the spleen, although bone marrow and lymph node uptake also occurs [14,15,23]. After administration *in vivo,* plasma proteins (opsonins) are absorbed onto the surface of liposomes, triggering recognition and liposome uptake by MPS cells through receptors such as the complement C3b receptor, the Fc receptor and others. [24-26].

Preparation of liposome

There are several approaches for medication of liposomes, which include the use of mechanical procedures, organic detergents, or through the junking of soap from phospholipid/ soap micelle fusions. In liposome medication, types and quantities of phospholipid, the ionic and charge parcels of waterless medium, as well as time hydrations, are important factors that determine the final liposome structure(27).

1. Multilamellar vesicles preparation:

Product of multilamellar vesicles is the simplest system in all liposome medications. In this system, stages of liposome generation are used as organic detergent for dissolving of lipid and drying of the redounded admixture. Combination of lipids similar as egg lecithin, cholesterol and phosphatidyl glycerol in a molar rate of0.91.00.1 are used independently. Chloroform or a admixture of chloroform and methanol in a typical rate of 21 are used independently. originally, each lipid element is dissolved in the organic detergent independently, followed by mixing in the suitable proportion with the other solubilized lipids to insure and invariant distribution of the lipids in admixture. latterly, nitrogen sluice is used to induce a film from the admixture in test tube. Also, in order to remove any last traces of organic detergent, the film of lipid is allowed to dry fully in an vacated chamber for a minimum of 4- 6 hours(28).

2. Unilamellar vesicles preparation:

The unilamellar vesicle is the most popular type of liposomes. Its liposome structure allows for an indeed distribution of trapped agents within a single internal waterless cube. There are several styles for medication of these structures including ultrasonication, extrusion through polycarbonate pollutants, snap- thawing, ethanol injection, soap system and medication of sterile large unilamellar vesicles. Bhatia et al(2015) used admixture of different small unilamellar vesicles(SUVs) populations for gain ternary GUV with invariant property(29).

3. Giant Unilamellar Liposomes Preparation:

There are so numerous styles in the medication of giant liposomes grounded on exercising only distilled water,non-electrolyte or zwitterions. There's an increase in magnet between membranes caused by the presence of ions conducting a net charge, and thereby inhibiting the separation of the membrane wastes during the rehydration and swelling process. lately, experimenters have demonstrated medication of giant liposomes, using physiological strength buffers. There are several styles for medication of these systems including electroformation, giant liposomes prepared in rapid-fire medication, using physiological buffer for medication of giant unilamellar liposomes and bibulous shock fashion(30). Also, Karamdad and associates(2015) used new system of a microfluidic for GUV medication and mechanical characterization(31).

Loading of Drugs by Liposomes

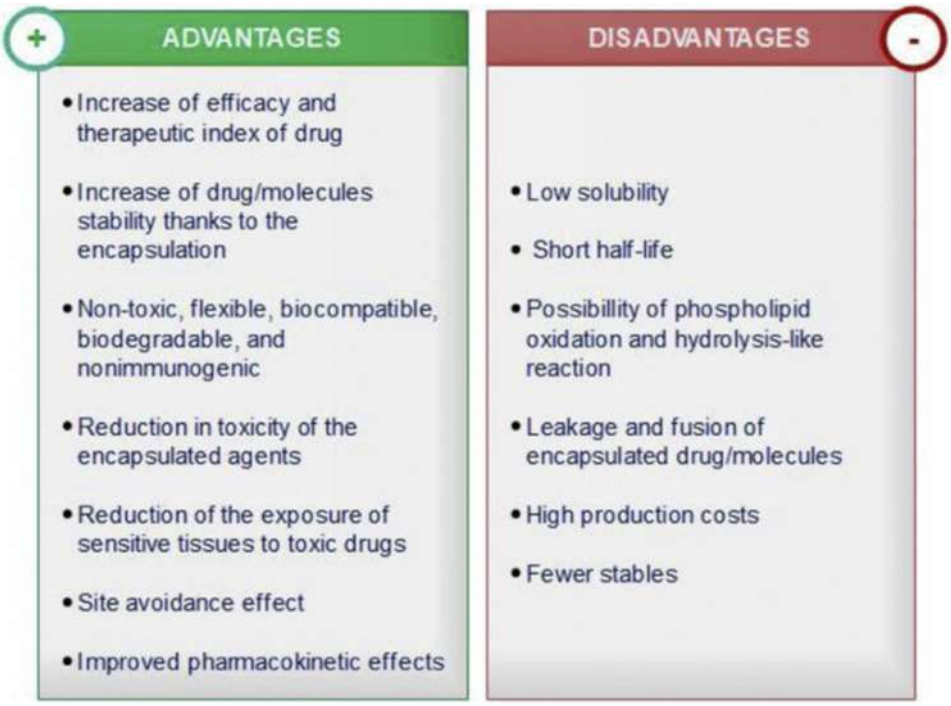
Encapsulation of Hydrophilic Drugs:

Encapsulation of hydrophilic medicines results in hydration of lipids hydrophilic medicines admixture. Through such a system, medicines can enter the liposome core and other accoutrements remain in outside part of the liposome. Remained accoutrements will remove medicine ruse in liposome. In order to purify these two corridor(medicines and remained outside accoutrements), gel filtration column chromatography and dialysis are used. In addition, dehumidification and rehydration system may be applied for high encapsulation of the DNA and proteins(32).

Encapsulation of Hydrophobic Drugs:

The phospholipid bilayer of liposomes is a region of hydrophobic medicine encapsulation. By ruse of this type of medicines(similar as verteporfin(Visudyne), movement of medicine will be be dropped towards the external waterless and inner corridor of liposomes. These medicines are reprised through solubilizing of medicine in the organic detergent and phospholipids. Region of medicine entrapement in liposome is the hydrophobic part of liposome. latterly, it's possible to use ray light for activation of medicine due to the treatment of wet macular degeneration(33).

Advantages and Disadvantages:



Applications of Liposomes

Liposomes provide superior therapeutic efficacy and safety as compared to existing formulations. Some of the major therapeutic applications of liposomes in drug delivery are as follows,

1) Site-avoidance delivery:

The cytotoxicity ofanti-cancer medicines to normal apkins is attributed to their narrow remedial indicator(TI). Under similar circumstances, the TI can be bettered by minimizing the delivery of medicine to normal cells by recapitulating in liposomes. Foreg. doxorubicin has a severe side effect of cardiac toxin, but when formulated as liposomes, the toxin was reduced without any change in the remedial exertion(38).

2) Site specific targeting:

Delivery of a larger bit of the medicine to the asked (diseased) point, reducing the medicine's exposure to normal apkins can be achieved by point specific targeting. On systemic administration, long circulating immunoliposomes are suitable to fete and bind to target cells with lesser particularity(39). Fore.g. in cases with intermittent osteosarcoma, there was an enhanced tumoricidal exertion of monocytes, when muramyl peptide derivations were formulated as liposomes and administered systemically.

3) Intracellular drug delivery:

Increased delivery of implicit medicines to the cytosol(where medicine receptors are present) can be fulfilled by using LDDS. N-(phosphonacetyl)-L- aspartate(PALA) is typically inadequately taken up into cells. similar medicines when reprised within liposomes, showed lesser exertion against ovarian excrescence cell lines in comparison to free medicine(40).

4) Sustained release drug delivery:

To achieve the optimum remedial efficacity, which requires a prolonged tube attention at remedial situations, liposomes give sustained release of target medicines(41). medicines like cytosine Arabinoside can be reprised in liposomes for sustained release and optimized medicine release rate in vivo.

5) Intraperitoneal administration:

Excrescences that develop in theintra-peritoneal(ip) depression can be treated by administering the medicine to ip depression. But the rapid-fire concurrence of the medicines from the ip depression results in minimized quantum of medicine at the diseased point. still, liposomal reprised medicines have lower concurrence rate, when compared to free medicine and can give a maximum bit of medicine in a prolonged manner to the target point(42).

Liposomes for Diagnostics and Therapeutic Applications

Liposomes are considerably employed in individual and remedial operations. Liposomes offer implicit advantages in the areas ofX-ray and glamorous resonance imaging, ultrasound and nuclear drug because of their high lading capacity, tolerability and towel selectivity and are more favored when compared to other forms of towel- picky discrepancy media(43). Medical imaging entails an applicable intensity of signal in order to distinguish certain structures from neighbouring apkins. Superparamagnetic liposomes are reported as largely effective glamorous resonance imaging(MRI) discrepancy agents. illustration, maghemite reprised liposomes synthesized from egg PC(phosphatidylcholine) and DSPEPEG(2000)(44). A number of liposome-grounded pharmaceutical products have been approved(Table 2). The success of liposome technology has spawned the growth of a " support assiduity " including outfit and excipient suppliers, as well as a number of biotechnology companies that concentrate specifically on the development of liposome-grounded medicinals.

Table 2. List of approved (marketed) liposomal drug products.

Product	Drug	Company	Indication target
Atragen™	Tretinoin	Aronex Pharmaceuticals Inc.	Acute promyelocytic leukemia
Amphotec	Amphotericin B	Sequus Pharmaceuticals Inc.	Fungal infections leishmaniasis
Ambisome™	Amphotericin B	NeXstar Pharmaceuticals Inc	Serious fungal infections
Amphocil™	Amphotericin B	Sequus Pharmaceuticals Inc	Serious fungal infections
Abelcet™	Amphotericin B	The Liposome Company.	Serious fungal infections
ALEC™	Dry protein free powder of DPPCPG	Britannia Pharm, UK	Expanding lung diseases in infants
Avian retrovirus vaccine	Killed avian retrovirus	Vineland lab, USA	Chicken pox
DaunoXome™	Daunorubicin citrate	NeXstar Pharmaceuticals Inc., USA	Kaposi sarcoma in AIDS
DepoDur	Morphine	Pacira Pharmaceuticals Inc	Post-surgical pain reliever
Daunoxome	Daunorubicin citrate	Galen Ltd	Kaposi sarcoma in AIDS
Depocyt	Cytarabine	Pacira Pharmaceuticals Inc	Treatment of lymphomatous meningitis
Doxil	Doxorubicin	Sequus Pharmaceuticals Inc.	Kaposi sarcoma in AIDS
Estrasorb	estradiol	Novavax	Menopausal Therapy
Evacet™	Doxorubicin	The liposome company, USA	Metastatic breast cancer
Epaxal –Berna Vaccine	Inactivated hepatitis-A Virions	Swiss serum & vaccine institute, Switzerland	Hepatitis A

Fungizone	Amphotericin B	Bristol-Myers Squibb, Netherland	Serious fungal infections
Mikasome®	Amikacin	NeXstar Pharmaceuticals Inc.	Bacterial infection
Nyotran™	Nystatin	Aronex Pharmaceuticals Inc.	Systemic fungal infections
Topex Br	Terbutaline sulphate	Ozone Pharmaceuticals Ltd.	Asthma
Ventus	Prostoglandin-E1	The liposome company	Systemic inflammatory disease
VincaXome	Vincristine	NeXstar Pharmaceuticals Inc.	Solid Tumors

New Generation of Liposomes

Liposomal drug delivery has created an opportunity to formulate a wide variety of difficult to deliver therapeutic agents. In spite of many products in the market and several others in the clinical trials, the instability of a drug during its transfer to the targeted site is still a problem. Therefore, to improve the drug stability, efficacy and to reduce the adverse effects by targeting the site of action, a new generation of liposomes have been explored using various phospholipids and their derivatives [45]. The new generation liposomes demonstrated considerable advantages with potential therapeutic benefits (Table **3**). However, still further investigation is needed to overcome the limitations encountered in terms of long term stability, entrapment efficiency and active targeting.

Table 3. New generation liposomes and their advantage

Type	Modification	Advantages
Archaeosomes	One or more lipids containing diether linkages	Highly stable liposomes
Niosomes	Non-ionic surfactant and cholesterol	Less susceptible to bile salts
Novasomes	Monoester of polyoxyethylene fatty acids, cholesterol and free fatty acids.Two to seven bilayer shells	High drug loading
Transfersomes	Lipid supramolecular aggregates	Highly flexible suitable for transdermal delivery
Ethosomes	Phospholipids and alcohol in relatively high concentration	More disruptive in the skin lipid bilayer organization suitable for transdermal delivery
Virosomes	Lipids surface modified with fusogenic viral envelope proteins	Intracellular delivery of antigens and DNA
Cryptosomes	Phospholipids and polaxamers or PEG	Improved stability
Emulsomes	Internal solid fat core surrounded by phospholipid bilayer	Suitable for encapsulation of hydrophobic drugs
Vesosomes	Multilamellar liposomes	Multidrug formulations are possible
Genosomes	Complex of cationic phospholipids and a functional gene or DNA	Suitable for gene delivery

Conclusions

The ability of liposomes to change the pharmacokinetics of their associated drugs and the biodistribution of the drug towards diseased tissues and away from normal tissues has led to considerable clinical benefit. Several liposomal drugs have been approved for clinical use and many more liposomal drug formulations, including anticancer, antibacterial and anti-inflammatory drugs, are in early-to-late clinical trials. For clinical applications where the strategy of passive targeting to tissues of increased vascular permeability would not be successful, e.g. in treating haematological malignancies, or in order to further increase the specific localisation of liposomal drugs to diseased cells, a number of laboratories are currently exploring ligand- or antibody-mediated targeted liposomal delivery systems.

References

1) Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface- active agents as observed in the electron microscope. *J Mol Biol*. 1964;8:660–668.

2) Bangham AD, Hill MW, Miller NG. Preparation and use of liposomes as models of biological membranes. In: Korn ED, editor. *Methods in Membrane Biology*. Vol 1. New York: Plenum; 1974:1–68.

3) Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, McCullough J. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomedicine*. 2013;9:1–14.

4) Felice B, Prabhakaran MP, Rodríguez AP, Ramakrishna S. Drug delivery vehicles on a nano-engineering perspective. *Mater Sci Eng C Mater Biol Appl*. 2014;41:178–195.

5) Fanciullino R, Ciccolini J. Liposome-encapsulated anticancer drugs: still waiting for the magic bullet? *Curr Med Chem*. 2009;16:4361–4373.

6) Valenzuela SM. Liposome techniques for synthesis of biomimetic lipid membranes. In: Martin DK, editor. Nanobiotechnology of biomimetic membranes. Boston, MA: Springer US; 2007. P. 75-87.

7) Gupta PK, Jaiswal AK, Kumar V, Verma A, Dwivedi P, Dube A, et al. Covalent functionalized selfassembled lipo-polymerosome bearing amphotericin b for better management of leishmaniasis and its toxicity evaluation. Mol Pharm 2014;11(3):951-63. doi: 10.1021/mp400603t

8) Euliss LE, DuPont JA, Gratton S, DeSimone J. Imparting size, shape, and composition control of materials for nanomedicine. *Chem Soc Rev*. 2006;35:1095–1104.

9) Papahadjopoulos D, Kimelberg HK. Phospholipid vesicles (liposomes) as models for biological membranes: their properties and interactions with cholesterol and proteins. In: *Progress in Surface Science*. Vol. Oxford: Pergamon; 1973:141–149.

10) Frolov VA, Shnyrova AV, Zimmerberg J. Lipid polymorphisms and membrane shape. *Cold Spring Harb Perspect Biol*. 2011;3:a004747.

11) Immordino ML, Dosio F, Cattel L. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. *Int J Nanomedicine*. 2006;1:297–315.

12) Betageri GV, Parsons DL. Drug encapsulation and release from multilamellar and unilamellar liposomes. *Int J Pharm.* 1992; 81:235–241.

13) Niven RW, Speer M, Schreier H. Nebulization of liposomes. II. The effects of size and modeling of solute release profiles. *Pharm Res.* 1991; 8:217–221.

14) Hwang KJ. Liposome pharmacokinetics. In: Ostro MJ, editor. Liposomes: from biophysics to therapeutics.New York: Marcel Dekker, 1987: 109-56

15) Allen TM, Hansen CB, De Menezes DEL. Pharmacokinetics of long circulating Iiposomes. Adv Drug Deliv Rev 1995; 16: 267-84

16) Gabizon A. Catane R, Uziely B. et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. Cancer Res 1994; 54: 987-92

17) Gill PS, Espina BM, Muggia F, et al. Phase VII clinical and pharmacokinetic evaluation of liposomal daunorubicin. J Clin Oncol 1995; 13: 996-1003

18) Cowens JW, Creaven PJ, Greco WR, et al. Initial clinical (phase I) trial ofTLC D-99 (doxorubicin encapsulated in Iiposomes). Cancer Res 1993; 53: 2796-802

19) Klibanov AL, Maruyama K. Torchilin VP. et al. Amphipathic polyethylene glycols effectively prolong the circulation time of Iiposomes. FEBS Lett 1990; 268: 235-7

20) Senior J, Delgado C, Fisher D, et al. Influence of surface hydrophilicity of liposomes on their interaction with plasma protein and clearance from the circulation: studies with poly(ethylene glycol)-coated vesicles. Biochim Biophys Acta 1991; 1062: 77-82

21) Allen TM, Hansen C. Pharmacokinetics of stealth versus conventional liposomes: effect of dose. Biochim Biophys Acta 1991; 1068: 133-41

22) Papahadjopoulos D, Allen TM, Gabizon A. et al. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. Proc Natl Acad Sci USA 1991; 88: 11460-4

23) Senior J, Crawley JC, Gregoriadis G. Tissue distribution of liposomes exhibiting long half-lives in the circulation after intravenous injection. Biochim Biophys Acta 1985; 839: 1-8

24) Alving CR. Wassef NM. Complement-dependent phagocytosis of Iiposomes: suppression by 'Stealth' lipids. J Liposome Res 1992; 2: 383-95

25) Aragnol D, Leserman L. Immune clearance of liposomes inhibited by an anti-Fc receptor antibody *in vivo*. Proc Nat! Acad Sci USA 1986; 83: 2699-703

26) Chonn A, Semple SC, Cullis PRo Association of blood proteins with large unilamellar liposomes *in vivo*. Relation to circulation lifetimes. J Bioi Chern 1992; 267: 18759-65

27) Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, et al. A review on phospholipids and their main applications in drug delivery systems. Asian J Pharm Sci 2015;10(2):81-98. doi: 10.1016/j.ajps.2014.09.004

28) Rieder AA, Koller D, Lohner K, Pabst G. Optimizing rapid solvent exchange preparation of multilamellar vesicles. Chem Phys Lipids 2015;186:39-44. doi: 10.1016/j.chemphyslip.2014.12.001

29) Bhatia T, Husen P, Brewer J, Bagatolli LA, Hansen PL, Ipsen JH, et al. Preparing giant unilamellar vesicles (GUVs) of complex lipid mixtures on demand: Mixing small unilamellar vesicles of compositionally heterogeneous mixtures. Biochim Biophys Acta 2015;1848(12):3175-80. doi: 10.1016/j.bbamem.2015.09.020

30) Motta I, Gohlke A, Adrien V, Li F, Gardavot H, Rothman JE, et al. Formation of giant unilamellar proteo-liposomes by osmotic shock. Langmuir 2015;31(25):7091-9. doi: 10.1021/acs.langmuir.5b01173

31) Karamdad K, Law RV, Seddon JM, Brooks NJ, Ces O. Preparation and mechanical characterisation of giant unilamellar vesicles by a microfluidic method. Lab Chip 2015;15(2):557-62. doi: 10.1039/c4lc01277a

32) Chatterjee SN, Devhare PB, Lole KS. Detection of negative-sense RNA in packaged hepatitis E virions by use of an improved strand-specific reverse transcription-PCR method. J Clin Microbiol 2012;50(4):1467-70. doi: 10.1128/JCM.06717-11

33) Verteporfin In Photodynamic Therapy Study Group. Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: Two-year results of a randomized clinical trial including lesions with occult with no classic choroidal neovascularization--verteporfin in photodynamic therapy report 2. Am J Ophthalmol 2001;131(5):541-60. doi: 10.1016/s0002- 9394(01)00967-9

34) Kulkarni R, Jaydeep D, Kumar A. Liposomes: A Novel Drug Delivery System. Int J Curr Pharm Res, 3(2):10- 18, (2011).

35) Herbert A. Lieberman, Martin M. Rieger Gilbert S. Banker, Ed. Liposomes. Pharmaceutical Dosage Forms: Disperse Systems, 2nd Edition. p. 45-83.

36) Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I. Preparation, Characterization and Applications of Liposomes: State of the Art. J Colloid Sci Biotechnol, 1(2):147-168, (2012).

37) Sayali V.Tarkunde, K.N. Gujar, Mallinath Harwalkar, M.S. Gambhire. Liposome – A Novel Drug Delivery. Int J Pure Appl Biosci, 2(6):92-102(2014).

38) Alyane M, Barratt G, Lahouel M (2016) Remote loading of doxorubicin into liposomes by transmembrane pH gradient to reduce toxicity toward H9c2 cells. Saudi Pharm J 24: 165-175.

39) Paszko E, Senge MO (2012) Immunoliposomes. Curr Med Chem 19: 5239- 5277.

40) Krieger ML, Eckstein N, Schneider V, Koch M, Royer HD, et al. (2010) Overcoming cisplatin resistance of ovarian cancer cells by targeted liposomes in vitro. Int J Pharm 0389: 10-17.

41) Loira-Pastoriza C, Todoroff J, Vanbever R (2014) Delivery strategies for sustained drug release in the lungs. Adv Drug Del Rev 75: 81-91.

42) Eloy JO, Claro de Souza M, Petrilli R, Barcellos JPA, Lee RJ, et al. (2014) Liposomes as carriers of hydrophilic small molecule drugs: Strategies to enhance encapsulation and delivery. Colloids and Surf B Biointerfaces 123: 345-63.

43) Sahoo SK, Labhasetwar V. Nanotech approaches to drug delivery and imaging. Drug Discov Today 2003; 8: 1112-20.

44) Laroui H, Rakhya P, Xiao B, Viennois E, Merlin D. Nanotechnology in diagnostics and therapeutics for gastrointestinal disorders. Diges Liver Dis 2013; 45: 995-1002

45) Çağdaş M, Sezer AD, Bucak S. Liposomes as Potential Drug Carrier Systems for Drug Delivery, Application of Nanotechnology in Drug Delivery (2014), PhD. Ali Demir Sezer (Ed.), ISBN: 978-953-51-1628 8, InTech, DOI: 10.5772/58459. (Available from: http://www.intechopen.com/books/applicationof-nanotechnology-in-drug-delivery/liposomes-aspotential-drug-carrier-systems-for-drug-delivery).