Journal of **Exploratory** Research in Pharmacology 2023 vol. 8(3) | 222–236
DOI: 10.14218/JERP.2022.00086

Review Article

Brief Insight on Nanovesicular Liposomes as Drug-delivery Carriers for Medical Applications

Madhavi Pravinkumar Chudasma, Sakshi Alpeshkumar Shah, Mohammad Hamran Nasiruddin Qureshi, Nirav Shah, Devarshi Shah, Riddhi Trivedi and Viral Hareshkumar Shah*

Department of Pharmaceutics, Sal Institute of Pharmacy, Ahmedabad, India

Received: November 16, 2022 | **Revised:** January 09, 2023 | **Accepted:** February 02, 2023 | **Published online:** April 27, 2023

Abstract

Nanotechnology has revolutionized and improved the potential of drug-delivery systems. Lipidic nanovesicles, termed liposomes, offer several benefits as drug-delivery carriers due to their compositional similarity with human biological membranes. Some of the advantages offered by liposomes include targeted drug delivery and improved pharmacokinetics of an entrapped drug substance, leading to enhanced bioavailability. Additionally, the reduction in the drug dose or dosing frequency, reduced drug toxicity or side effects, longer and controlled therapeutic response of a drug essential for the treatment of chronic diseases, and improved patient acceptance margins are some other benefits offered by liposomes. The easily modifiable surface of liposomes makes it an ideal vehicle for targeting a drug to the desired site. The current review provides insight on liposomes as drug-delivery carriers. This review summarizes the classification of compositional constituents of liposomes based on their chemical nature and structure. Morphology-based classification of liposomes along with methods of preparation and characterization for liposomes are also summarized. The current review emphasizes the medical applications of liposomes, specifically as delivery carriers for therapeutic and diagnostic agents. The article features detailed therapeutic applications of liposomes based on routes of administration and specific disease conditions. Diagnostic applications of liposomes for improving the efficiency of available techniques to treat diseases such as cancer are also discussed. Liposomes are thus reviewed as multifaceted nanovesicular carriers with potential therapeutic and diagnostic medical applications. The prospective multifunctional application of combining imaging functionality with therapeutic agents in a single liposome for diagnosis and real-time treatment is anticipated to be the future of liposomal formulations.

Introduction

The efficacy of a drug substance for the treatment, mitigation, management, or prevention of disease conditions or disorders relies upon its therapeutic efficiency, low exhibition of toxic or side effects at the required therapeutic dose or dosing frequency, and ability to reach the desired target site at the effective concentration as well as to stay at that target site for the desired time length. Due to restrictions in allowed structural modifications of drug substances required to achieve the desired pharmacokinetic properties while maintaining its therapeutic action, the role of drug deliverables is prominent and indispensable for achieving the desired therapeutic response of drug substances in low doses, for longer duration, and with reduced side effects. Nanotechnology has revolutionized the potential of drug deliverables in delivering the drug substances at the desired target site in therapeutic doses with improved pharmacokinetic properties of adsorption, distribution, metabolism, and excretion, which ultimately affect the bioavailability of drug substances. Reduction in drug toxicity or side effects by decreasing its dose or dosing frequency, targeting the drug to the desired site, controlling the action of drug substances desired to treat chronic disease or disorders, and improving patients' compliance are some added benefits of nanotechnology. Amongst the nanoparticle drug-delivery carriers, vesicular drug-delivery systems, particularly lipid-based vesicular systems that encapsulate the drug substance in the core or within the matrix of phospholipids, are gaining attention because of several of their advantages, which are compiled and summarized in the current review paper.

The first and most widely explored lipid vesicle-based nanodrug-delivery carrier is popularly known as liposomes. The notion of liposomes, which were initially known to be "bangosomes," was

Keywords: Vesicular drug-delivery system; Nanocarriers; Liposomes; Phospholipids; Therapeutic applications; Diagnostic applications.

Abbreviations: BBB, blood-brain barrier; PDI, polydispersity index.

***Correspondence to:** Viral Hareshkumar Shah, Department of Pharmaceutics, Sal Institute of Pharmacy, Ahmedabad 380060, India. ORCID: https://orcid.org/0000-0003-4740-0856. Tel: +91-9277202747, Fax: +91-079-67129000, E-mail: viralshah779@yahoo.com

How to cite this article: Chudasma MP, Shah SA, Qureshi MHN, Shah N, Shah D, Trivedi R, *et al*. Brief Insight on Nanovesicular Liposomes as Drug-delivery Carriers for Medical Applications. *J Explor Res Pharmacol* 2023;8(3):222–236. doi: 10.14218/JERP.2022.00086.

pioneered by Alec D. Banhgam between the 1950s and 1960s, and they received recognition as drug-delivery carriers in 1971 by the scientist Gregory Gregoriadis. Liposomes, which are composed of closed bilayers of phospholipids to form a vesicle, exhibit significant structural and compositional similarities to biological membranes prevailing in the human body; thus, liposomes enhance the adsorption rate and bioavailability of drug substances entrapped in their vesicular structure. In addition, the easily modifiable surface properties of liposomes make it a potential drug-delivery vehicle for achieving targeted therapeutic activity of drug substances. Currently, liposomal formulations of antifungal agents and anticancer chemotherapeutic agents for tumor targeting have exhibited considerable benefits over conventional therapies. More recently, mucormycosis, a fatal fungal infection, has been demonstrated to be managed significantly better through liposomal delivery of amphotericin B (antifungal drug substance) in comparison to any other drug-delivery system.[1] The potential of liposomes has been explored and proven in medical as well as in nonmedical fields like catalysts, bioreactors, ecology, cosmetics, etc.[2]

Composition of liposomes

Phospholipids

Phospholipids are the building block and basic structural component of liposomes. A bilayer of phospholipids constitutes the shell of vesicular liposomes. Based on the desired properties and applications, different types and classes of phospholipids are used for formulating liposomes.

Classification of phospholipids based on their internal composition (alcohol active group)

Glycerophospholipids are phospholipids with a glycerol backbone, comprised of glycerol-3-phosphate esterification at the C1 and C2 positions. There are six different subtypes of glycerophospholipids, including phosphatidic acid, lecithin (phosphatidylcholine), cephalin (phosphatidylethanolamine), phosphatidylinositol, phosphatidylserine, and cardiopoline (diphosphatidylglycerol).[3]

Phosphatidic acid is the smallest and most basic glycerol phospholipid. It has a strong anion that provides a negative charge density to the membrane. Phosphatidic acid is a synthetic phospholipid with several possible derivatives (Table 1), which can be synthesized according to the required application.[4]

Lecithin is an egg yolk-derived natural choline-based phospholipid. It is a zwitterionic molecule, consisting of partial positive and negative charges. This phospholipid has a major influence on membrane properties such as "fluidity." There are two types of lecithin: dipalmitoyl lecithin and lysolecithin. The structures and chemical names of several lecithin derivatives synthesized according to their applications are depicted in Table 1.

The naming of cephalins was derived from the word cephalic, meaning "related to the head." The polar head group of cephalins is highly reactive and small in size; therefore, this type of compound has a low hydration rate. Cephalins are synthesized by adding cytidine diphosphate ethanolamine to diglyceride. Cephalins can be treated with ethanolamine, which consists of a reactive nitrogen base, to further synthesize several other derivatives for varied applications. Several derivatives of cephalins are as follows: 1,2-dierucoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine, and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine.

Phosphatidylinositol is synthesized by the reaction between the isomeric form of myo-inositol and phosphatidic acid. Inositol is present in all types of tissues as well as in cells and has a saturated fatty acid chain. Phosphatidylinositol is anionic in nature.

Phosphatidylserine is a synthetic phospholipid. Serine is anionic in nature, and derivatives of phosphatidylserine include the following: 1,2-dilauroyl-*sn*-glycero-3-phosphoserine, 1,2-dimyristoyl-*sn*-glycero-3-phosphoserine, 1,2-dioleoyl-*sn*-glycero-3-phosphoserine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoserine, and 1,2-distearoyl-*sn*-glycero-3-phosphoserine.

Cardiolipin contains two molecules of phosphatidic glycerol. It consists of two optically active carbons and also has a number of unsaturated fatty acid chains. Several derivatives of cardiolipin are as follow: 1,2-dierucoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)], 1,2-dilauroyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)], 1,2-dilauroyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)] (ammonium salt)], 1,2-dimyristoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)], 1,2-dimyristoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)] (ammonium salt)], 1,2-dioleoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)], 1,2-dipalmitoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)], 1,2-dipalmitoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)] (ammonium salt), 1,2-distearoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)], 1,2-distearoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol) (ammonium salt)], and 1-palmitoyl-2-oleoyl-*sn*-glycero-3 [phospho-rac-(1-glycerol)].

Chemically, sphingomyelins are *N*-acyl sphingosine-1-phosphatidylcholine. The backbone of sphingomyelins is sphingosine. Each molecule of sphingomyelin consists of an acyl chain. Generally, naturally obtained sphingosines contain 20 or more acyl groups. Therefore, the structure of sphingomyelin molecules is asymmetric. The phase-transition temperature of sphingomyelins ranges from 30°C to 45°C.

Classification of phospholipids based on surface charges

Anionic phospholipids are negatively charged phospholipids that resemble the biological membrane proteins. Phosphatidic acid, phosphatidylinositol, and phosphatidylserine are some examples of anionic phospholipids.

Cationic phospholipids are positively charged phospholipids that help to deliver negatively charged nucleic acids like DNA, mRNA, and siRNA by ionic interactions. Dioleoyl phosphatidylethanolamine and 1,2-dioleoyl-3-trimethylammonium propane are two examples of cationic phospholipids.

Classification of phospholipids based on its origin source

Natural phospholipids are obtained from natural sources like wheat germ, flax seed, sunflower, soybeans, egg yolk, and milk. Meanwhile, synthetic or semisynthetic phospholipids are tailor made, chemically synthesised phospholipids. Some examples of synthetic phospholipids are 1,2-dimyristoyl-*sn*-glycero-3-phosphate, 1,2-dipalmitoyl-*sn*-glycero-3-phosphate, dipalmitoylphosphatidylcholine, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine, dimyristoylphosphatidylglycerol, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol, sodium salt, etc.

Classification of phospholipids based on the fatty acid chain saturation

Saturated chain fatty acids exhibit comparatively more chemical stability against oxidation and hydrolysis. Some examples of saturated fatty acids include 1,2-dimyristoyl-*sn*-glycero-3-phosphate,

Table 1. Phospholipids used in liposomal formulations along with the chemical name and structure[4]

Phospholipid	Structure and chemical name	
Phosphatidic acid	1,2-Dioleoyl-*sn*-glycero-3-phosphate (DOPA)	1,2-Dilauroyl-*sn*-glycero-3-phosphate (DLPA)
	1,2-Dimyristoyl-*sn*-glycero-3-phosphate (DMPA)	1,2-Distearoyl-*sn*-glycero-3-phosphate (DSPA)
	1,2-Dipalmitoyl-*sn*-glycero-3-phosphate (DPPA)	
Lecithin	1,2-Didecanoyl-*sn*-glycero3-phosphocholine (DDPC)	1,2-Dierucoyl-*sn*-glycero-3-phosphocholine (DEPC)
	1,2-Dilinoleoyl-*sn*-glycero3-phosphocholine (DLOPC)	1,2-Dilauroyl-*sn*-glycero-3-phosphocholine (DLPC)
	1,2-Dimyristoyl-*sn*-glycero3-phosphocholine (DMPC)	1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)
	1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC)	1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC)
	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC)	1,2-Dierucoyl-*sn*-glycero-3-phosphocholine (DEPC)
Cephalin (phosphatidyl ethanolamine)	1,2-Dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE)	1,2-Dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE)
	1,2-Dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE)	1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE)
	1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE)	1,2-Dierucoyl-*sn*-glycero-3-phosphoethanolamine (DEPE)
	1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine (DSPE)	
Cardiolipin	1,2-Dierucoyl-*sn*-glycero-3[phospho-rac-(1-glycerol) (DEPG)	1,2-Dilauroyl-*sn*-glycero-3[phospho-rac-(1-glycerol) (ammonium salt) (DLPG-NH_4)
	1,2-Dimyristoyl-*sn*-glycero-3[phospho-rac-(1-glycerol) (DMPG)	1,2-Dioleoyl-*sn*-glycero-3[phospho-rac-(1-glycerol) (DOPG)
	1,2-Dipalmitoyl-*sn*-glycero-3[phospho-rac-(1-glycerol) (ammonium salt) (DPPG-NH_4)	1,2-Distearoyl-*sn*-glycero-3[phospho-rac-(1-glycerol) (ammonium salt) (DSPG-NH_4)
	1-Palmitoyl-2-oleoyl-*sn*-glycero-3[phospho-rac-(1-glycerol) (POPG)	
Phosphatidyl serine (PS)	1,2-Dilauroyl-*sn*-glycero-3-phosphoserine (DLPS)	1,2-Dimyristoyl-*sn*-glycero-3-phosphoserine (DMPS)
	1,2-Dioleoyl-*sn*-glycero-3-phosphoserine (DOPS)	1,2-Dipalmitoyl-*sn*-glycero-3-phosphoserine (DPPS)
	1,2-Distearoyl-*sn*-glycero-3-phosphoserine (DSPS)	

1,2-dipalmitoyl-*sn*-glycero-3-phosphate, 1,2-distearoyl-*sn*-glycero-3-phosphate, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine, and 1,2-dilauroyl-*sn*-glycero-3-phosphoethanolamine, etc.

Unsaturated chain fatty acids are more prone to oxidation and hydrolysis. Some examples of unsaturated fatty acids are; 1,2-dierucoyl-*sn*-glycero-3-phosphocholine, 1,2-dilinoleoyl-*sn*-glycero-3-phosphocholine, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine, 1,2-dierucoyl-*sn*-glycero-3-phosphocholine, etc.

Classification of phospholipids based on morphology (shape)

Phospholipids can be classified based on their morphology as follows: conical shaped, e.g., lysophosphatidylcholine and lysophosphatidic acid; straight chain, e.g., phosphatidylcholine; and inverted conical shaped, e.g., phosphatidic acid and phosphatidylethanolamine.

Cholesterol

Along with phospholipids, cholesterol is an important component of liposomes. It plays an important role in holding the tight vesicular structure of liposomes thereby maintaining vesicular integrity and stability. Due to its structural composition, cholesterol occupies the gaps between phospholipids in the vesicles, thereby preventing diffusion and leakage of entrapped components. Additionally, cholesterol can also increase the loading efficacy of both hydrophilic and lipophilic materials to be incorporated in liposomes.

Types of liposomes

Liposomes are categorized into different types mainly based on parameters like size of the vesicle, lamellarity, and composition of vesicles (Table 2).

Lamellarity-based classification

The number of bilayers present in liposomes determines the lamellarity of the vesicles. It is an important parameter that affects the

Table 2. Classification of liposomes based on size, lamellarity, and composition

Parameter	Size	Lipid bilayer
Size and lamellarity		
Small lamellar vesicles	20–100 nm	Single
Medium lamellar vesicles	>100 nm	Single
Large lamellar vesicles)	>100 nm	Single
Oligo lamellar vesicles	0.1–1 nm	5 lipid bilayers
Multilamellar vesicles	>0.5 µm	5–25 lipid bilayers
Multivesicular vesicles	>1 mm	multi compartment (honeycomb like)
Application and composition		
Conventional liposomes		
Charged liposome		
Stealth stabilized liposomes		
Stimuli-responsive liposomes		
Actively-targeted liposomes		

encapsulation efficiency of liposomes. Based on lamellarity, the vesicles are classified as unilamellar vesicles (consisting of one phospholipid bilayer), oligolamellar vesicles (consisting of five phospholipid bilayers), and multilamellar vesicles (consisting of 5–25 phospholipid bilayers).

Size-based classification

The vesicle dimension is an acute parameter affecting the circulation half-life of liposomes in the human body. Based on the size of the liposomes, they are classified as small vesicles (with a vesicular diameter between 20 nm and 100 nm), medium vesicles (with a vesicular diameter >100 nm), or giant vesicles (with a vesicular diameter of 1 µm).[5]

Liposomes are often classified based on a mixture of size and lamellarity parameters as follows: small unilamellar vesicles, with a diameter of 20–100 nm and one lipid bilayer; medium unilamellar vesicles, with a diameter of >100 nm and containing one lipid bilayer; giant unilamellar vesicles, with a diameter of 1 µm and containing one lipid bilayer; oligolamellar vesicles, with a diameter of 0.1–1 nm and containing approximately five lipid bilayers; multilamellar large vesicles, with a diameter of >0.5 µm and containing 5–25 lipid bilayers; and multivesicular vesicles, liposomes with a diameter of >1 mm and containing a multicompartment (like a honeycomb) of lipid bilayers, e.g., vesicles within vesicles.[5]

Application- and composition-based classification

Conventional liposomes are liposomes that are usually composed of solely phospholipids and/or cholesterol. They are vesicular structures with lipid bilayers surrounding an aqueous core; they exhibit a brief blood circulation time due to the speedy uptake by the mononuclear phagocyte system, which in turn helps in macrophage-targeting and vaccination purposes.[6]

Charged liposomes are physically stabilized liposomes as the surface charges on the liposomes repel each other and decrease the aggregation rate and extent. Positively charged cationic liposomes avert their passive diffusion into cells; therefore, they are broadly used in delivering macromolecules for gene therapy, tumor-targeted therapy, and brain-targeted therapy by crossing the blood-brain barrier (BBB). Anionic liposomes are negatively charged and are commonly utilized for transdermal drug transport due to their greater permeation rate across the stratum corneum.[4]

Stealth liposomes are surface-modified liposomes with a polymeric, specific ligand and polyethylene glycol (PEG) that are made for specific applications like targeting, stabilization, or an increased residence time in the human body.

Stimuli-responsive liposomes are liposomes that are activated by physicochemical or biochemical stimuli for targeting a specific site or for achieving a desired encapsulated drug release. Stimuli-responsive liposomes have two induction sources: external induction sources (like heat, magnetic field, light, electric field, and ultrasound) and internal induction sources (like pH, redox potential, and enzymes).

Actively-targeted liposomes are liposomes that are useful for the site-specific delivery of drugs through receptor targeting, monoclonal antibodies, or large or small protein-based targeting or peptide-based targeting molecules.

Mechanism of action of liposomes

Endocytosis

The liposome is engulfed by the cell membrane after binding to the cell surface through a receptor, ligand, or antibody. Liposomes release the entrapped drug after cellular engulfment; thus, first-pass metabolism of the drug is prevented.

Content transfer

Liposomes have a similar structure to human cell membranes, so the phospholipids of liposomes transfer their contents (drug substance) across the cell membrane after interacting with the cell membrane lipids.

Fusion

In this mechanism, the phospholipids of liposomes fuse with the phospholipids of the cell membrane, and during this fusion mechanism, the cell membrane and liposome lipids coalesce together in a single membrane, resulting in drug content transfer from the liposomes to the cells.

Adsorption

In this process, liposomes adhere to the surface of the cell membrane through attractive forces between the liposomes and the cell membrane, followed by the release of drug from the liposomes and the uptake of unchanged drug by the cells.

Methods for preparing liposomes

For liposomal formulation preparation, the following generic steps are followed:[4–8] (1) lipids are dissolved in an organic solvent; (2) drug loading (active or passive); (3) evaporation of the organic solvent of the lipidic solution; (4) hydration of the lipid; (5) downsizing the vesicles; (6) post formulation process (purification, sterilization); (7) liposome characterization.

The most widely used techniques for liposome preparation are summarized below.

Bangham method (lipid film hydration)

The phospholipid components are dissolved in an organic solvent, and the organic solvent is gradually evaporated under a vacuum to obtain a uniform, thin lipid film of stacked phospholipid bilayers. The formed film is finally hydrated with water or an aqueous buffer to obtain liposomes.[4]

Solvent injection method

Phospholipids are dissolved in ethanol and injected into preheated aqueous buffer or distilled water. In a preheated aqueous phase, ethanol is evaporated and the dispersed phospholipids self-assemble to form closed vesicles called liposomes. Alternatively, phospholipids can be dissolved in ether, which is a more volatile organic solvent, and then the ether can be evaporated more effectively to form concentrated liposomes with a high entrapment efficiency.[8]

Reverse-phase evaporation method

The reverse-phase evaporation method is based on the principle of inverted micelle formation through reverse-phase evaporation. In this technique, phospholipids are dissolved in an organic phase, and aqueous components of liposomes are dissolved in a water phase. The formation of inverted micelles is the foundation of reverse-phase evaporation. Both the phases are then macerated together at a high temperature, leading to evaporation of the organic solvent and the formation of liposomes.[8]

Detergent removal method

Phospholipids are solubilized at their critical micelle concentrations with the aid of detergents to form micelles. The formed micelles acquire themselves into closed vesicles as the detergent is removed through dialysis using detergent-free buffers or through gel permeation chromatography. In this technique, when detergent and phospholipids are added to an aqueous phase, the micellar size and polydispersity increase significantly, and dilution of the system beyond the micellar phase boundary transit the polydisperse micelles to more uniform vesicles.

Once the liposomal vesicles are obtained by utilizing any of the abovementioned techniques, the desired size and lamellarity of the liposomes can be obtained using downsizing techniques such as sonication (probe or bath), extrusion (membrane or French cell), freeze thawing, etc.[8]

Physical characterization of liposomes

The quality, efficiency, and stability of liposomes as a drug-delivery formulation can be assessed through several characterization tests, as mentioned below:

Particle size, polydispersity index (PDI), lamellarity, and zeta potential

The particle size, lamellarity, and zeta potential properties depend on the composition components of the liposomes and the preparation method. The particle size, zeta potential, and PDI are important characterization parameters that have an impact on the physicochemical stability, entrapment efficiency, *in-vivo* performance, and heterogeneity of the liposomal formulation. The particle size, PDI, and zeta potential of liposomes can be determined by dynamic light-scattering techniques and laser Doppler velocimetry. Electron microscopy (e.g., scanning electron microscopy and transmission electron microscopy), fluorescence microscopy, and atomic force microscopy-based techniques can be used to assess the lamellarity and morphology of liposomes.[9] Liposomal formulations with PDI values less than 0.5 and a zeta potential of around +30 ± 5 mV and −30 ± 5 mV are considered as monodispersed liposomes with a good physicochemical stability.

Entrapment efficiency

The maximum amount of drug that can be encapsulated in a liposomal vesicle is termed as its entrapment efficiency. The entrapment efficiency of liposomes is determined by disruption of the phospholipid bilayer using methanol, ethanol, or triton X-100, followed by centrifugation and decantation of the entrapped drug in pellet form. The entrapped drug pellets are then solubilized in a suitable solvent and quantified using spectrophotometric or chromatographic techniques.

Drug release

The composition, method of preparation, lamellarity, and surface modifications of liposomes may alter the release rate of an entrapped drug substance from liposomes. The dialysis technique is frequently used to assess the *in-vitro* release rate of a drug substance from a liposomal formulation. Prewetted dialysis bags with specific molecular weight cut-off values that can retain the liposomes while allowing the drug released from the liposomes to cross the membrane and enter the receptor solvent medium are used. At predetermined time points, aliquots from the receptor solvent medium are withdrawn and quantified using spectrophotometric or chromatographic techniques to determine the release rate of drug from the liposomal formulation.

Physicochemical stability

Phospholipids are susceptible to a number of chemical reactions like peroxidation, hydrolysis, and photolysis, leading to their deterioration and thereby impacting their chemical stability. The ability of liposomes to retain their particle size and PDI is termed as its physical stability. Inappropriate particle sizes and surface charges may lead to liposome aggregation, thereby reducing their physical stability, which can significantly impact their performance. In the presence of serum proteins or other biological substances, the ability of liposomes to retain their integrity is referred to as biological stability. The physicochemical stability of liposomes can be assessed as per the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines.

In addition to the abovementioned tests, liposomes are assessed for other parameters like pH, osmolarity, pyrogenicity, sterility, toxicity, pharmacokinetics, and other evaluation parameters depending on the final dosage form into which liposomes are dispersed.

Applications of liposomes

Therapeutic applications as drug-delivery carriers

Liposomes are drug-delivery vesicles with varied applications due to the advantages offered by them. Liposomes can increase the efficacy of a drug substance by improving its pharmacokinetic parameters. Liposomes, due to their morphology, composition, and flexibility in terms of surface modifications can offer several benefits for delivering the entrapped drug substance effectively via various drug-delivery routes. Liposomes are beneficial drug-delivery carriers specifically for drugs with a narrow therapeutic index because they aid drug distribution and increase drug safety and efficacy. Treatment strategies are experiencing a paradigm shift from synthetic drug molecules to biologically derived therapeutics.[10] Liposomes also have been widely investigated as potential delivery carriers for therapeutic biologics, e.g., antisense oligonucleotides, cloned genes, recombinant proteins, therapeutic peptides (hormones), nucleic acids (siRNA, mRNA, pDNA), CRISPR/Cas9, and vaccines.

Application of liposomes as drug-delivery carriers administered via different routes

Liposomes have the potential to improve the efficacy of routes like ocular, nasal, inhalation, oral, transdermal, and rectal as portals to enable the drug substance to reach the systemic circulation or target sites (Table 3).[11–33]

Ocular route

Various drugs used in ophthalmic disorders that are meant to be delivered to the systemic circulation through the ocular route suffer the limitation of a low effectiveness when delivered through conventional ophthalmic formulations like eye drops.[34] The low residence time of a drug in the eye due to rapid drainage through the lacrimal fluid as well as the low permeability of most drugs result in very low ophthalmic bioavailability. Liposomes, due to their composition, increase the drug substance permeation rate and extent across the ocular surface, resulting in an improved ophthalmic bioavailability and thereby therapeutic efficiency of the drug substance. Additionally, drug-loaded liposomes entrapped or dispersed in ocular lenses can prolong the residence time of drugs in the ophthalmic cavity. Charged liposomes administered as an ophthalmic spray or as a hydrogel have the ability to adhere with mucin present on the corneal surface through electrostatic bond formation, thereby increasing the residence time of drug in the ophthalmic cavity; moreover, charged liposomes can also control the release rate of entrapped drug for a prolonged therapeutic response.[35] Liposomes adsorb to the corneal layer and transfer the drug molecules to the corneal epithelial cell membrane. Liposomes can also travel by liposomal endocytosis. Liposomes consisting of a hydrogel or spray exhibit a higher penetration than conventional ophthalmic formulations.[36]

Intranasal route

The intranasal route is one of the most prominent routes for the delivery of drugs that cannot be administered through the oral route due to limitations like first-pass metabolism, instability of biological conditions of the human gastrointestinal tract, the local action of the drug is needed in the nasal cavity, or targeting the drug to the brain.[37] Liposomes are considered as biocompatible drug-delivery carriers, which enhance the absorption of drugs across the nasal mucosa. Additionally, liposomes offer added advantages like the ability to encapsulate both small and large drug molecule with a broad range of hydrophobicity levels and pKa values, thus providing controlled and targeted drug release profiles. Several successful liposomal formulations for enhancing the nasal penetration and absorption of drug molecules like calcitonin, insulin, and influenza vaccine are approved by the US Federal Drug Administration and commercially available in the global market. Liposome-loaded nasal hydrogel or mucoadhesive gel formulations can increase the nasal drug penetration rate because of the longer duration of residence time of the drug substance at the absorption site. Liposomal nasal hydrogels can also control the release of the drug to achieve an efficient prolonged therapeutic response. The intranasal route is also considered as a promising route for rapid drug absorption into the brain via the systemic circulation.[38,39] Nasal cavities can also transport a drug substance to the brain through olfactory neurons or the geminal nerve. Two major barriers for transporting drug substances to the brain are the cerebrospinal fluid barrier and the BBB. Rapid mucociliary clearance in the central nervous system decreases the drug absorption. The liposomal drug-delivery system would not only overcome the mentioned barriers, but it would also increase the absorption rate of the drug substance through the nasal cavity due to its phospholipid composition.[40]

Inhalation route

Chronic and acute respiratory infections are difficult to treat through conventional inhalation-based antibiotic treatment therapy. The low tissue/cell penetration rate of antibiotics delivered through the conventional inhalation route in the form of dry powder inhalers requires high doses of antibiotics, leading to dose-related drug toxicity or antibiotic resistance. Inhaled liposomes are a novel approach for treating respiratory infections. Liposomal inhalation preparations are biocompatible, nontoxic, biodegradable, and stable in the inhibitory barrier of the sputum. Additionally, inhalable drug-loaded liposomes can increase the therapeutic index, drug absorption and penetration rates, and retention time of the drug at targeted sites, thereby maximizing the therapeutic efficacy and minimizing the drug side effects. Conventional inhalation antibiotics exhibit a short half-life in alveoli, so three or more daily administrations of drug are required.[41] The liposomal inhalation drug formulation can release the drug in a slow and controlled manner to achieve prolonged drug action, which in turn would decrease the dosing frequency of the drug substance by maintaining the concentration of the drug in the lungs above the minimum effective concentration.

Oral route

Liposomes were conventionally designed for parenteral drug administration, but they were then modified and investigated to increase the oral bioavailability of macromolecules like insulin by increasing the absorption rate of insulin through the gastrointestinal tract, resulting in destruction of the phospholipid bilayers and leading to leakage of the entrapped drug substance.[42] The hurdles of the oral administration of liposomes can be combated through surface modification or surface coating of liposomes and by substitution of phospholipids with hydrogenated phospholipids. Coating liposomes with large-chain polymers like chitosan can impart stability to liposomes in a gastric environment. Matrix modifications of liposomes by adding bile salts also improve the stability of liposomes in the gastrointestinal tract.[43] Colon targeting of a drug-delivery substance through the oral route is essential to reduce the toxicity and to enhance the efficacy of therapeutic agents used for the treatment of local colonic diseases or disorders like colon cancer, inflammatory bowel disease, and colonic infection.[44]

Table 3. Representative illustrations of investigated liposomal drug formulations administered through various routes[11–33]

Route of drug administration	Liposomal composition	Therapeutic agent	Application
Ocular	Phosphatidylcholine, cholesterol	Acyclovir	Increased absorption and controlled release of the drug
	1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), cholesterol	Lidocaine	Controlled and prolonged release of the drug for 8 days
	Phosphatidylcholine, cholesterol	Ciprofloxacin	Liposomal hydrogel increases ocular availability of the drug, enhances the therapeutic efficacy, and prolongs the release of the drug
	L-phosphatidylcholine, cholesterol	Acetazolamide	Multilamellar liposomes provide a significant decrease in the intraocular pressure for a prolonged duration
	Egg phosphatidylcholine, cholesterol	Pilocarpine nitrate	Increased drug bioavailability
Nasal	L-α-Dipalmitoylphosphocholine (DPPC), cholesterol	Acyclovir	Enhanced nasal penetration and drug bioavailability
	L-alpha-Phosphatidyl choline, cholesterol	Ovalbumin (OVA)	Increased stability, bioavailability, and therapeutic efficacy of ovalbumin
	1,2-Dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy (polyethy leneglycol)-2000 (DSPE-PEG-2000) and cholesterol	Protamine	Improved and potent immunotherapeutic effect against cancer
	1,2-ditetradecanoyl-*sn*-glycero-3-phosphocholine, cholesterol	Fexofenadine	Increased drug bioavailability and prolonged drug exposure
	Soybean phosphatidylcholine, cholesterol	Valproic acid	Increased permeation of valproic acid across the blood-brain barrier, sustained release of drug, reduced drug side effects, and improved patient compliance
Inhalation	1, 2-Dipalmitoyl-*sn*-glycero-3-phosphoglycerol-sodium salt (DPPG)/1, 2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)/1, 2- distearoyl-snglycero-3-phosphocholine (DSPC)/1, 2-Distearoyl-*sn*- glycero-3- phosphoglycerol, sodium salt (DSPG)/1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (DPPE), cholesterol	*N*-acetylcysteine	Coated liposomes resulted in the prolonged release of drug, good deposition and retention of coated liposomes in the lungs
Oral	1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)/1,2-dipalmitoylsn-glycero-3-phosphoethanolamine-*N*-[4-(*p*-maleimidomethyl) cyclohexane-carboxamide] (DPPE-MCC)/fluorescence labelled phospholipid 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (DOPE-Liss.Rhod.)	Salmon calcitonin	Improved oral bioavailability and stability of the drug substance
	Dipalmitoyl phosphatidylcholine, phosphatidylcholine, phosphatidylinositol	Insulin	Improved oral bioavailability and stability of the drug substance
	Phosphatidylcholine, cholesterol	Paclitaxel	Increased drug uptake by the gastrointestinal mucosa and improved drug intestinal absorption
	L-a-Dipalmitoylphosphatidyl choline (DPPC), cholesterol	Cyclosporine A (CSA)	Improved bioavailability of CSA
	1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC)	Tamoxifen citrate and Raloxifene hydrochloride	Enhanced oral absorption of both drug substances with increased tumor targeting and anti-cancer properties of the drug substances

(continued)

Table 3. *(continued)*

Route of drug administration	Liposomal composition	Therapeutic agent	Application
Transdermal	1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), cholesterol	Indocyanine green (ICG)	Chitosan coating of liposomes stabilized the encapsulated unstable photosensitizer, ICG. In addition, positively-charged chitosan coating of liposomes facilitated the cellular uptake and permeation of ICG
	Soya phosphatidylcholine (PC), 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine	Methotrexate (MTX)	Enhanced transdermal flux, targeting of drug to the epidermal and dermal sites, controlled release of MTX
	Soyaphosphatidylcholine (PC), cholesterol	Propranolol hydrochloride	Improved transdermal flux and bioavailability of the drug substance
	Hydrogenated soya phosphatidyl choline (HSPC), cholesterol	Acyclovir sodium	Improved systemic bioavailability of the drug substance
	Soya phosphatidylcholine (soya PC)	Indinavir	Improved entrapment efficiency, enhanced transdermal flux, and improved systemic bioavailability of Indinavir
Rectal	1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (DSPE-PEG2000)	Doxorubicin (Dox)	Improved anticancer efficacy and reduced side effects of Dox
	Phosphatidylcholine (PC) phosphatidylethanolamine, cholesterol	Ferritin	Increased immunization efficacy of ferritin against sexually transmitted infections such as HIV

A surface-modified coated liposomal formulation can also be useful for targeting the drug substance to a specific colonic tumor site to increase the therapeutic efficacy and to reduce the associated side effects of drug substances.

Transdermal route

The stratum corneum layer of the skin acts as a major barrier towards permeation of a drug substance to the underlying epidermal, dermal, or subcutaneous regions or to the systemic circulation via the skin. High-molecular-weight and hydrophilic drug substances have a very poor permeation rate and a low systemic bioavailability when delivered through the transdermal route. Due to their structural and compositional similarities to the cell membranes and skin layers, liposomal drug-delivery systems can easily permeate across the stratum corneum barrier layer of the skin along with the encapsulated drug substance.[45] Thus, liposomes can increase the permeation rate and therefore the bioavailability of drugs delivered through the transdermal route.

Rectal route

The rectal administration of a drug is considered in medical practice for drug substances against a local disease or disorder as well as for increasing the systemic bioavailability of therapeutic agents that are metabolized or are unstable when delivered through oral or other routes. The major limitations associated with delivering a drug substance through the rectal route are as follows: low mucosal permeation rate of the drug substance and degradation of the drug substance by enzymes present in the microflora of the rectal cavity. Liposomes are stable in the mucosa and can increase the permeation rate of the drug substance across the rectal mucosa and also protect the encapsulated drug against enzymatic degradation, thereby increasing the systemic bioavailability of drug substances delivered through the rectal route.

Parenteral route

Liposomes are used as formulations for delivering drugs through the parenteral route to accomplish therapeutic goals like improving the therapeutic efficiency, reducing the toxicity, and sustaining or controlling the release rate of a drug substance for a prolonged duration by creating a depot system. Liposomes offer several benefits such as adaptability towards the delivery of macromolecules, capability of encapsulating both hydrophilic and lipophilic drug substances, targeted delivery to a desired site, and adaptability towards surface modification, enabling an increase in its residence time in systemic circulation.[46] Thus, liposomes can serve as potential formulations for delivering a drug through the parenteral route.

Disease-specific applications of liposomes

Skin disorders

Mostly, skin disorders arise superficially on the surface of the human body. Based on the involvement and location of the disorder, liposomal formulations offer site-specific drug delivery and can deliver the drug to different layers of the skin, ranging from the topical surface, epidermis, dermis, or deep down in blood vessels beneath the skin.[11] Topical liposomal formulations avoid the first-pass metabolism of the drug and provide site-specific drug delivery (Table 4).[12,47–51]

Acne

Visible nodules on the face can be termed as acne vulgaris. Come-

Table 4. Representative illustrations of investigated liposomal drug formulations used to treat skin disorders[47–51]

Disease	Liposome composition	Therapeutic agent	Application
Acne	Cholesterol HSPC	Adapalene	Topical adapalene liposome delivery enhanced drug permeation and reduced adapalene side effects
Skin burns	Lipoid S 100	Mupirocin	Liposomal formulation dispersed in hydrogel showed sustained drug release and improved antimicrobial activity of drug
Vitiligo	Cholesterol, DC-cholesterol, sodium deoxycholate	Psoralen	Psoralen loaded deformable liposomal vesicles showed high penetration rate of drug and reduced the pigmentation process
Psoriasis	Phosphatidyl choline, oleic acid	Methotrexate	Methotrexate loaded liposomes exhibited higher skin permeability
Leishmaniasis	Cholesterol, DPPC, DPPS	Lupane	Lupane-loaded liposomes efficiently diminished parasite abidance affecting certain cytokines

DC, dimethylaminoethane-carbamoyl; DPPC, dipalmitoylphosphatidylcholine; DPPS, dipalmitoylphosphatidylserine; HSPC, hydrogenated soy phosphatidylcholine.

dones, seborrhoea, lifeless pores due to plugged hair follicles filled with oil are common causes of acne. Retinoids, antibiotics, and several herbal agents are used to treat acne.[13] Liposomal formulations can increase the penetration rate of the drug substance in the superficial layers of the skin or to the systemic circulation through blood vessels located deep down in the skin as desired according to the mechanism of action of the drug substance, thereby improving its therapeutic efficiency.[14]

Skin burns

Skin burns may occur due to various causes such as chemical exposure, electric shock, and exposure to heat or radiation. According to the depth of the wound and the area of the injury, a skin burn can be characterized as one of four different degrees.[15] Liposomal formulations encapsulating growth factors essential for tissue regeneration enhance the wound healing process.[16]

Vitiligo

Visible white patches on the skin due to melanocyte loss is termed as vitiligo. Phototherapy, surgical methods, and topical and systemic drug treatments are the preferred methods to treat vitiligo.[17] A topical liposomal formulation with an encapsulated drug substance has been demonstrated to increase the efficacy of a drug substance by increasing or enhancing the repigmentation process.[18,19]

Psoriasis

A chronic autoimmune skin disorder associated with epidermal hyperproliferation, abnormal differentiation of keratinocytes, leukocyte infiltration, and endothelial vascular changes is termed as psoriasis. According to the severity of disease, the treatment strategy is chosen.[20,21] A liposomal formulation can increase the therapeutic efficiency of drugs like cyclosporine used to treat psoriasis by improving its permeation rate across the skin layers, thereby increasing the therapeutic drug concentration at the desired site.[22]

Leishmaniasis

Leishmaniasis is a parasitic disease that can be treated through local or systemic chemotherapy based on the severity of the disease.[23] Conventional drug therapy includes antimonials, amphotericin B, pentamidine, paromomycin, and miltefosine, which are less effective and exhibit high toxicity as well as several adverse reactions.[20–23] Drug-loaded liposome-based therapy enhances the drug permeation rate and can sustain the release of drug for a prolonged therapeutic effect.[24] Liposomal formulations can decrease the dose or dosing frequency of a drug substance due to the increased therapeutic concentration of the drug reaching the target site, thereby decreasing the dose- and dosing frequency-related side effects and adverse reactions.

Autoimmune disorders

Autoimmune disorders are chronic conditions caused due to genetic, environmental, or physiological factors.[25–27] A liposome-based nanovesicle drug-delivery approach is noninvasive, can increase the therapeutic efficiency of a drug substance, can increase patient compliance, can be delivered site-specifically, and hence is beneficial for the effective treatment of autoimmune disorders (Table 5).[28–30,52–54]

Table 5. Representative illustrations of investigated liposomal drug formulations used to treat autoimmune disorders[52–54]

Disease	Liposome composition	Therapeutic agent	Results
Type 1 diabetes	Cholesterol, SPC, DSPE-biotin	Insulin	Improved insulin oral bioavailability and accelerated cellular uptake
Multiple sclerosis	EPC, ManDOGa (targeting ligand)	Immunodominant peptides of MBP	MBP delivered through liposomes efficiently suppressed autoimmune encephalomyelitis in Dark Agouti rats, lowering the severity of the attack
Rheumatoid arthritis	Cholesteryl hemisuccinate, DPPE, DPPE-HA	Prednisolone	Liposomes increased the cellular uptake and cytostatic activity of entrapped prednisolone

DSPE, 1,2-distearoyl-*sn*-glycero-3-phosphorylethanolamine; DPPE, dipalmitoylphosphatidylethanolamine; HA, hyaluronic acid; EPC, egg phosphatidylcholine; ManDOG, monomannosyl dioleyl glycerol; MBP, myelin basic protein; SPC, soybean phosphatidylcholine.

Table 6. Representative illustrations of investigated liposomal drug formulations used to treat respiratory disorders[57–60]

Disease	Liposome composition	Therapeutic agent	Results
Asthma	Cholesterol, DOPS	rSLPI	rSLPI-loaded liposomes enhanced the drug stability and its residence time in the lungs
Pneumonia	Cholesterol, HSPC, DSPE-PEG	Daptomycin, Clarithromycin	The combined drug-liposomal formulation exhibited more effective antibacterial activity in comparison to conventional formulations
Pneumonia	Cholesterol, DPPC	Polymyxin B	The liposomal formulation increased drug uptake by macrophages and delivered polymyxin B into the epithelial lining fluid, ensuring a greater drug effect at the target site
Tuberculosis	Cholesterol, lecithin	Rifampicin	The rifampicin-loaded liposomal formulation reduced the drug toxicity, enhanced the drug bioavailability, and released the drug in a controlled manner

DOPS, dioleoyl[phospho-L-serine]; DPPC, dipalmitoylphosphatidylcholine; DSPE-PEG, distearoylphosphoethanolamine-*N*-poly(ethylene glycol) 2000; DPPC, dipalmitoylphosphatidylcholine; HSPC, hydrogenated soy phosphatidylcholine; rSLPI, recombinant secretory leukocyte protease inhibitor.

Type 1 diabetes

Type 1 diabetes is a metabolic disease in which pancreatic insulin-excreting β-cells are permanently demolished.[31,32] Therapy for this disease includes insulin injected daily; however, patient compliance is often poor.[33,47,48] The oral route is not viable due to the poor bioavailability of insulin.[49–51,55] Liposomal formulations can increase the stability and therefore the bioavailability of insulin delivered through the oral route, leading to the effective treatment of type 1 diabetes.

Multiple sclerosis

Multiple sclerosis is an inflammatory neurological condition. This disease is characterized by demyelinated axons and oxidative stress. In the treatment of multiple sclerosis, the major challenge is for the drug to cross the BBB and reach the target site. Liposomal formulations can improve the biodistribution and pharmacokinetics of the drug, leading to an enhanced permeation rate of drug across the BBB.

Rheumatoid arthritis

Rheumatoid arthritis is a chronic autoimmune disease condition of the bone joint with cartilage destruction, leading to pain and tissue dysfunction. Rheumatoid arthritis therapy includes treatment through nonsteroidal anti-inflammatory drugs, biologics, corticosteroids, and disease-modifying antirheumatic drugs. However, the drugs exhibit a poor systemic bioavailability and a rapid clearance rate, leading to high doses or dosing frequency, several side effects, and poor patient compliance.[56] Liposomal delivery can enhance the systemic bioavailability of drugs due to an increased permeation rate, leading to a reduced dose and/or dosing frequency as well as fewer drug-related side effects.

Respiratory disease

A liposome-based nanovesicular drug-delivery system can provide enhanced therapeutic efficacy in the treatment of respiratory disease due to the higher site specificity and enhanced dose accuracy at the target sites, thus reducing systemic toxicity and irritation in the lungs (Table 6).[52,53,57–60]

Asthma

Asthma is mainly an allergic response and a chronic condition leading to airway inflammation and blockage. β2 agonists, corticosteroids, anticholinergics, and oxygen therapy are used for the management and treatment of asthma. Liposomes can increase the residence time of drugs in the lungs and therefore increase the therapeutic efficacy of the drug substances.[56]

Pneumonia

Pneumonia is an infection of the air sac in a single lung or both lungs caused by fluid or pus deposition. Antibiotics are prescribed for the treatment and management of pneumonia. Liposomes can increase the residence time of drugs in the lungs as well as the lung tissue concentration of the drug substance, thus increasing the therapeutic efficacy.[57–59]

Tuberculosis

Tuberculosis is an airborne pulmonary infection. Antituberculosis agents like isoniazid, ethambutol, rifampin, and pyrazinamide are used for the treatment and management of tuberculosis. A liposomal formulation of antituberculosis agents may enhance the pulmonary drug concentration, leading to a reduction in the dose and dosing frequency as well as resulting in a reduced incidence of drug resistance.[60]

Neurological disorders

The BBB offers a major challenge to drug delivery to the brain in neurological disorders as it is highly lipophilic in nature and has a high expression of efflux pump proteins like P-glycoprotein, which decreases the penetration rate of drug substances across the BBB. Due to the lipophilic nature of liposomes, they can prove effective in transporting drugs to the brain by overcoming the BBB (Table 7).[63–67]

Alzheimer's disease

Alzheimer's disease is a neurodestructive disease state caused by the formation of β-amyloid plaques. Alzheimer's disease leads to memory loss and brain atrophy. Surface-modified novel liposomal formulations can easily transport the drug across the BBB to the target site, thus increasing the therapeutic efficiency of a drug substance in Alzheimer's disease patients.[61]

Seizures

A central nervous system disease caused by unusual neuronal signals in the brain leads to seizures. The lipophilic nature of a liposomal formulation efficiently delivers the drug to the target location, leading to rapid and effective seizure management.

Table 7. Representative illustrations of investigated liposomal drug formulations used to treat neurological disorders[63–67]

Disease	Liposome composition	Therapeutic agent	Results
Alzheimer's disease	Cholesterol, DSPC, DSPE-PEG, transferrin	α-Mangostin	Surface modified liposomes with transferrin resulted in enhanced BBB penetration and brain-targeting drug delivery
Seizure	Cholesterol, SPC	Nimodipine	Nimodipine-loaded liposomal formulation increased the bioavailability of drug and reduced the pilocarpine-induced seizure attack in mice model
PD	Cholesterol, PC, chitosan	Levodopa	Chitosan-coated levodopa liposomes diminished levodopa-induced dyskinesia and increased the therapeutic efficiency of levodopa
Migraine	Soya lecithin	Rizatriptan benzoate	improved drug permeation, higher flux across nasal mucosa, no damage to epithelial layer, improvement in bioavailability
CI	DPPC, DSPE-PEG	Tacrolimus	Liposome formulation lowered brain injury and increased the therapeutic efficacy of tacrolimus

BBB, blood-brain barrier; DSPC, distearoylphosphocholine; DSPE-PEG, distearoylphosphoethanolamine-*N*-poly(ethylene glycol) 2000; DPPC, dipalmitoylphosphocholine; PC, phosphatidylcholine.

Parkinson's disease

Parkinson's disease is a neuron deterioration disease condition caused by the absence of dopamine in the basal ganglia and also by the loss of dopaminergic neurons. A liposomal formulation can target the delivery of a drug that is essential in the treatment of Parkinson's disease to the brain, thus leading to less neuronal loss.[62]

Cerebral infarction

Cerebral infarction is a brain tissue disease caused by the reduction of blood flow as well as the depletion of nutrients and oxygen supply to the brain tissues, and it eventually leads to brain cell death. The early management and effective treatment of cerebral infarction reduce the risk of brain hemorrhage. Liposomes can increase the bioavailability and therapeutic efficiency of drugs used for the effective management and rapid treatment of cerebral infarction.

Cardiac disorders

Nanoliposomal formulations enhance the plasma residence time and drug accumulation at the target site, thus increasing the therapeutic efficiency of drug substances used to treat cardiac disorders. Additionally, the surface modification of liposomes can aid targeted drug delivery and control the release of drug substances used to treat cardiac disorders (Table 8).[69–71]

Atherosclerosis

Atherosclerosis is caused by lipid accumulation, which in turn results in narrowing blood vessels and a poor blood flow in the artery, leading to myocardial infarction, stroke, and angina.[68] Surface-modified liposomal vesicles with encapsulated antiatherosclerotic agents can enhance the targeted action of a drug at the plaque formation site, thereby increasing the therapeutic effect of the drug.[69]

Myocardial infarction

Myocardial infarction is caused by a poor blood supply in the heart muscles. A liposomal formulation can target the drug substance to the heart and result in its accumulation in the infarcted region, inflicting fewer myocardial infarctions and cardiac fibrosis areas, thus improving the drug substance efficiency and reducing its side effects.[70]

Restenosis

Restenosis is a condition in which there is the narrowing of arteries, leading to a restricted blood flow. A liposomal formulation can control the release of the drug, leading to the prolonged therapeutic response of the drug substance, which is desired in restenosis.[71]

Cancer

Abnormal and uncontrolled multiplication of cells leading to tumor formation is termed as cancer. The treatment and management of cancer is done mainly through surgery or chemotherapy, which is very painful due to biodistribution and the effect of anticancer agents not only to tumor cells but also to healthy cells. Anticancer chemotherapy lacks specificity towards the target site, has a low plasma retention time, exhibits severe toxic side effects, and is not very stable or permeable.[72] Anticancer drug-loaded liposomes can

Table 8. Representative illustrations of investigated liposomal drug formulations used to treat cardiac disorders[69–71]

Disease	Liposome composition	Loaded drug	Results
Atherosclerosis	Cholesterol, DPPC, DSPE-PEG-E-selectin binding peptide	Atorvastatin	Atorvastatin and curcumin-loaded liposomes with pegylation reduced drug toxicity and inflammatory reactions of drug; also, pegylation of liposomes with selectin binding peptide resulted in enhanced cellular uptake of drug-loaded liposomes
Myocardial infarction	Cholesterol, lecithin, sodium deoxycholate	α-tocopherol	α-Tocopherol-loaded liposomal hydrogel increased the stability and therapeutic efficacy of α-tocopherol
Restenosis	EPC, DOTAP, DSPE-PEG	Estradiol	Liposomal formulations resulted in the controlled release and increased therapeutic efficacy of estradiol

DOTAP, dioleoyltrimethylammoniumpropane; DPPC, dipalmitoylphosphocholine; DSPE-PEG, distearoylphosphoethanolamine-*N*-poly(ethylene glycol) 2000; EPC, egg phosphatidylcholine.

Table 9. Representative illustrations of investigated liposomal drug formulations used to treat cancer[72–74]

Disease	Liposome composition	Loaded drug	Results
AIDS-related Kaposi's sarcoma	Cholesterol, PEGylated phoshpholipids	Doxorubicin	Surface modified liposomes prolonged the half-life and anticancer efficiency of doxorubicin
Lung cancer	Cholesterol, PEGylated phoshpholipids	Etopside	Liposomal formulation enhanced cell apoptosis induction, increased cell cycle arrest and exhibited enhanced antiproliferative effect
Pancreatic cancer	Cholesterol, PEGylated phospholipids	Azobenzene derivatives	Liposomal formulation diminished the nonspecific biodistribution of anticancer drugs along with controlled release drug delivery and reduced the multidrug resistance of drug

be targeted specifically to tumor sites through proper surface modifications, reducing the impact of chemotherapy on healthy cells and thereby reducing the associated pain. Additionally, liposomes have the ability to enhance permeability, impart biocompatibility, reduce immunogenicity, and increase the retention of drug at the target site; thus, they act as a potential carrier for chemotherapeutic agents (Table 9).[72–74]

Infectious disease

Fungal and viral infections

Recently, in 2021, people worldwide were affected by the COVID-19 pandemic. A current study has illustrated the effective creation of a remdesivir dosage form that is nebulized and scalable for self-medication against COVID-19. AL-Rem (aerosolized remdesivir) is practical for large-scale manufacturing thanks to the use of Federal Drug Administration-approved phospholipids and a modified hydration technique with a widely utilized particle size reduction technology.[75] Some COVID-19 patients also were infected by black fungi, leading to the infectious disease termed as mucormycosis. Amphotericin B is used as the first-line treatment for mucormycosis. Amphotericin B in its free form has a poor penetration rate at the target location, leading to its decreased efficiency and increased dose or dosing frequency and thus resulting in dose-related side effects like ionosphere toxicity and neurotoxicity.[76] A liposomal amphotericin B formulation demonstrated an improved drug penetration rate and uptake at the target site, resulting in an increased therapeutic efficiency, a reduced drug dose, fewer dose-related side effects, and less drug toxicity.[77] A liposomal formulation of amphotericin B also has been reported to reduce the incidence of drug resistance.[1]

Parasitic and bacterial infections

Malaria is endemic in several nations worldwide, and the major limitations of antimalarial drug substances are treatment failure due to *Plasmodium* species resistance as well as limited bioavailability due to poor water solubility, biostability, and permeability. A drug-delivery platform using liposomes can overcome the limitations of antimalarial therapy as liposomes, due to their dual hydrophilic as well as hydrophobic nature, can deliver water-soluble and lipid-soluble antimalarial drugs together to reduce the incidence of *Plasmodium* species resistance. Liposomes additionally offer the benefits of biodegradability, biocompatibility, and low toxicity, making them a potential vehicle for delivering antimalarial drug substances. Liposomes serve as an excellent drug-delivery carrier in parasitic infections like Leishmaniasis. Liposomes can offer targeted therapy to specific and targeted sites desired in parasitic infections, thereby increasing the therapeutic efficacy and reducing the toxicity of drug substances as well as the incidence of drug resistance.

Diagnostic applications of liposomes

In addition to their wide therapeutic applications, liposomes also have been investigated and exploited as carriers of chemical substances used for diagnostic purposes. Due to the inherent photoimaging properties of the components utilized in liposomal formulations, they can be utilized in various bioimaging techniques such as photoacoustic imaging and fluorescence.[78] Additionally, since the lipid components of liposomes can interchange with serum lipoproteins, the serum lipoproteins have a decreased ability to absorb imaging agents. Also, liposomes can improve the solubility and bioavailability of encapsulated imaging agents, thereby improving their imaging performance.[79] Furthermore, liposomes have the ability to increase the sensitivity of detection as they can bind to specific cells through receptor-mediated endocytosis due to the low-density lipoproteins that migrate within the liposomes.

In cardiovascular disorders, liposomes have been explored to improve the sensitivity and specificity of ultrasonography and echocardiography for investigating thrombosis diagnosis and for thrombolytic therapy monitoring.[80]

Liposome lipids conjugated with fluorescent labels have been demonstrated to be a useful technique to visualize the physical positions of antigens and to understand the mechanics of the major histocompatibility complex pathway in phagocytic antigen-presenting cells, which would be helpful for successful and effective vaccine development.[81] Thus, liposomes can serve as a potential delivery carrier for increasing the diagnostic efficiency of available imaging techniques. Also, several research investigations have explored the multifunctional potential of liposomes by combining their imaging functionality with therapeutic agents in a single liposome for diagnosis and real-time treatment.[82] Representative illustrations of commercialized Federal Drug Administration-approved therapeutic liposomal formulations are depicted in Table 10.

Future perspectives

Liposomes are multifaceted nanovesicular drug-delivery carriers with potential therapeutic and diagnostic medical applications. Liposomes are drug-delivery vehicles that have the potential to increase the therapeutic efficiency of a drug substance by offering benefits like targeted delivery at the desired site, enhanced bioavailability, and reduced dose/dosing frequency. The prospective multifunctional application of combining imaging functionality with therapeutic agents in a single liposome for diagnosis and real-time treatment is anticipated to be the future of liposomal formulations.

Conclusions

Liposomes as nanosized lipidic vesicular carriers have been used in a broad range of medical applications. The potential of li-

Table 10. Representative illustrations of commercialized Federal Drug Administration-approved therapeutic liposomal formulations

Brand name	Active pharmaceutical ingredient	Indication	Company
Doxil®	Doxorubicin	AIDS-related Kaposi's sarcoma	Baxter Healthcare Corp.
AmBisome®	Amphotericin B	Febrile fungal infection, leishmaniasis, candida, and cryptococcus infection	Galen Pharma
DepoCyt®	Cytarabine	Lymphomatous meningitis	Pacira Pharmaceuticals
DaunoXome®	Daunorubicin	HIV-related Kaposi sarcoma	Galen Pharma
DepoDur®	Morphine	Pain management	Pacira Pharmaceuticals
Exparel®	Bupivacaine	Postsurgical analgesia	Pacira Pharmaceuticals
Marqibo®	Vincristine	Acute lymphoblastic leukemia	Acrotech Biopharma
Onivyde®	Irinotecan	Adenocarcinoma of the pancreas	Ipsen Inc.
Inflexal®	Inactivated hemaglutinin of influenza virus strain A and B	Influenza	Janssen vaccines

posomes as drug-delivery carriers for a broad range of therapeutic substances, ranging from biologicals to synthetic drugs, is promising as they increase the therapeutic efficiency of drugs. Liposomes offer benefits such as targeted drug delivery to the disease location, improved pharmacokinetic parameters and bioavailability of the drug substance, reduced toxicity and side effects of the drug substance, and increased circulation residence time of the entrapped drug in the blood. Due to the inherent photoimaging properties of the components utilized in the liposomal formulation, they exhibit diagnostic applications in various bioimaging techniques like photoacoustic imaging and fluorescence. Liposomes can improve the solubility and bioavailability of encapsulated imaging agents, thereby improving the imaging performance of several diagnostic techniques. Liposomes also have been investigated for multifunctional applications by combining their imaging functionality with therapeutic agents in a single liposome for diagnosis and real-time treatment. Thus, liposomes have been proven as lipid vesicular carriers with potential in a wide range of medical applications, and future investigations and developments will broaden their scope as delivery vesicles in multifaceted areas.

Funding

The authors received no funding for completing this review.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Contributed to study concept and design, supervision, and critical revision of the manuscript (VHS), drafting (MPC, SAS, MHNQ), supervision (NS, DS, RT).

References

[1] Fisher EW, Toma A, Fisher PH, Cheesman AD. Rhinocerebral mucormycosis: use of liposomal amphotericin B. J Laryngol Otol 1991;105(7):575–577. doi:10.1017/s0022215100116652, PMID:1875144.

[2] 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.

[3] Shah V, Jobanputra A, Saxena B, Nivsarkar M. Development and Characterization of Saturated Fatty Acid-Engineered, Silica-Coated Lipid Vesicular System for Effective Oral Delivery of Alfa-Choriogonadotropin. AAPS PharmSciTech 2021;22(3):118. doi:10.1208/s12249-021-01985-0, PMID:33782790.

[4] Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: structure, composition, types, and clinical applications. Heliyon 2022;8(5):e09394. doi:10.1016/j.heliyon.2022.e09394, PMID:35600452.

[5] Dymek M, Sikora E. Liposomes as biocompatible and smart delivery systems - the current state. Adv Colloid Interface Sci 2022;309:102757. doi:10.1016/j.cis.2022.102757, PMID:36152374.

[6] Lee MK. Liposomes for Enhanced Bioavailability of Water-Insoluble Drugs: In Vivo Evidence and Recent Approaches. Pharmaceutics 2020;12(3):264. doi:10.3390/pharmaceutics12030264, PMID:32183185.

[7] Lee W, Im HJ. Theranostics Based on Liposome: Looking Back and Forward. Nucl Med Mol Imaging 2019;53(4):242–246. doi:10.1007/s13139-019-00603-z, PMID:31456856.

[8] 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.

[9] Kanasova M, Nesmerak K. Systematic review of liposomes' characterization methods. J Monatsh Chem 2017;148:1581–1593. doi:10.1007/s00706-017-1994-9.

[10] Kim EM, Jeong HJ. Liposomes: Biomedical Applications. Chonnam Med J 2021;57(1):27–35. doi:10.4068/cmj.2021.57.1.27, PMID:33537216.

[11] Law SL, Huang KJ, Chiang CH. Acyclovir-containing liposomes for potential ocular delivery. Corneal penetration and absorption. J Control Release 2000;63(1-2):135–140. doi:10.1016/s0168-3659(99)00192-3, PMID:10640587.

[12] Gulsen D, Li CC, Chauhan A. Dispersion of DMPC liposomes in contact lenses for ophthalmic drug delivery. Curr Eye Res 2005;30(12):1071–1080. doi:10.1080/02713680500346633, PMID:16354620.

[13] Hosny KM. Ciprofloxacin as ocular liposomal hydrogel. AAPS PharmSciTech 2010;11(1):241–246. doi:10.1208/s12249-009-9373-4, PMID:20151337.

[14] Durrani AM, Davies NM, Thomas M, Kellaway IW. Pilocarpine bioavailability from a mucoadhesive liposomal ophthalmic drug delivery system. J Int Pharmaceutics 1992;88(1-3):409–415. doi:10.1016/0378-5173(92)90340-8.

[15] Alsarra IA, Hamed AY, Alanazi FK. Acyclovir liposomes for intranasal systemic delivery: development and pharmacokinetics evaluation. Drug Deliv 2008;15(5):313–321. doi:10.1080/10717540802035251, PMID:18763162.

[16] Kaplan M, Demiröz FT, Vural I, Çelebi N. Development and characterization of gels and liposomes containing ovalbumin for nasal delivery.

J Drug Delivery 2018;44:108–117. doi:10.1016/j.jddst.2017.12.006.
[17] Mai Y, Guo J, Zhao Y, Ma S, Hou Y, Yang J. Intranasal delivery of cationic liposome-protamine complex mRNA vaccine elicits effective anti-tumor immunity. Cell Immunol 2020;354:104143. doi:10.1016/j.cellimm.2020.104143, PMID:32563850.
[18] Jufri M, Yuwanda A, Surini S, Harahap Y. Study of valproic acid liposomes for delivery into the brain through an intranasal route. Heliyon 2022;8(3):e09030. doi:10.1016/j.heliyon.2022.e09030, PMID: 35284670.
[19] Hamedinasab H, Rezayan AH, Mellat M, Mashreghi M, Jaafari MR. Development of chitosan-coated liposome for pulmonary delivery of N-acetylcysteine. Int J Biol Macromol 2020;156:1455–1463. doi:10.1016/j.ijbiomac.2019.11.190, PMID:31770553.
[20] Hathout RM, Mansour S, Mortada ND, Guinedi AS. Liposomes as an ocular delivery system for acetazolamide: in vitro and in vivo studies. AAPS PharmSciTech 2007;8(1):1. doi:10.1208/pt0801001, PMID:17408209.
[21] Qiang F, Shin HJ, Lee BJ, Han HK. Enhanced systemic exposure of fexofenadine via the intranasal administration of chitosan-coated liposome. Int J Pharm 2012;430(1-2):161–166. doi:10.1016/j.ijpharm.2012.04.007, PMID:22525082.
[22] Gradauer K, Barthelmes J, Vonach C, Almer G, Mangge H, Teubl B, *et al*. Liposomes coated with thiolated chitosan enhance oral peptide delivery to rats. J Control Release 2013;172(3):872–878. doi:10.1016/j.jconrel.2013.10.011, PMID:24140721.
[23] Kisel MA, Kulik LN, Tsybovsky IS, Vlasov AP, Vorob'yov MS, Kholodova EA, *et al*. Liposomes with phosphatidylethanol as a carrier for oral delivery of insulin: studies in the rat. Int J Pharm 2001;216(1-2):105–114. doi:10.1016/s0378-5173(01)00579-8, PMID:11274812.
[24] Liu Y, Yang T, Wei S, Zhou C, Lan Y, Cao A, *et al*. Mucus adhesion- and penetration-enhanced liposomes for paclitaxel oral delivery. J Int J Pharm 2018;537(1-2):245–256. doi:10.1016/j.ijpharm.2017.12.044.
[25] Meshal MAA, Khidr SH, Bayomi MA, Angary AAA. Oral administration of liposomes containing cyclosporine: a pharmacokinetic study. J Int Pharmaceutics 1998;168(2):163–168. doi:10.1016/S0378-5173(98)00066-0.
[26] Mutlu Ağardana NBM, Değimb Z, Yılmazc S, Altıntaş L, Topale T. Tamoxifen/raloxifene loaded liposomes for oral treatment of breast cancer. J Drug Deliv Sci Techno 2020;57:101–612. doi:10.1016/j.jddst.2020.101612.
[27] Lee EH, Lim SJ, Lee MK. Chitosan-coated liposomes to stabilize and enhance transdermal delivery of indocyanine green for photodynamic therapy of melanoma. Carbohydr Polym 2019;224:115143. doi:10.1016/j.carbpol.2019.115143, PMID:31472877.
[28] Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. J Control Release 2007;123(2):148–154. doi:10.1016/j.jconrel.2007.08.005, PMID:17884226.
[29] Mishra D, Garg M, Dubey V, Jain S, Jain NK. Elastic liposomes mediated transdermal delivery of an anti-hypertensive agent: propranolol hydrochloride. J Pharm Sci 2007;96(1):145–155. doi:10.1002/jps.20737, PMID:16960826.
[30] Jain SK, Gupta Y, Jain A, Rai K. Enhanced transdermal delivery of acyclovir sodium via elastic liposomes. Drug Deliv 2008;15(3):141–147. doi:10.1080/10717540801952407, PMID:18379926.
[31] Dubey V, Mishra D, Nahar M, Jain V, Jain NK. Enhanced transdermal delivery of an anti-HIV agent via ethanolic liposomes. Nanomedicine 2010;6(4):590–596. doi:10.1016/j.nano.2010.01.002, PMID: 20093197.
[32] Lokerse WJM, Kneepkens ECM, Hagen TLMT, Eggermont AMM, Grüll H, Koning GA, *et al*. In depth study on thermosensitive liposomes: Optimizing formulations for tumor specific therapy and in vitro to in vivo relations. Biomaterials 2016;82:138–150. doi:10.1016/j.biomaterials.2015.12.023, PMID:26761778.
[33] Zhou F, Kraehenbuhl JP, Neutra MR. Mucosal IgA response to rectally administered antigen formulated in IgA-coated liposomes. Vaccine 1995;13(7):637–644. doi:10.1016/0264-410X(94)00029-M, PMID:7668033.
[34] 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.
[35] Craig JP, Purslow C, Murphy PJ, Wolffsohn JS. Effect of a liposomal spray on the pre-ocular tear film. Cont Lens Anterior Eye 2010;33(2):83–87. doi:10.1016/j.clae.2009.12.007, PMID:20096622.
[36] Achouri D, Alhanout K, Piccerelle P, Andrieu V. Recent advances in ocular drug delivery. Drug Dev Ind Pharm 2013;39(11):1599–1617. doi:10.3109/03639045.2012.736515, PMID:23153114.
[37] Pallagi E, Jójárt-Laczkovich O, Németh Z, Szabó-Révész P, Csóka I. Application of the QbD-based approach in the early development of liposomes for nasal administration. Int J Pharm 2019;562:11–22. doi:10.1016/j.ijpharm.2019.03.021, PMID:30877028.
[38] Vyas SP, Goswami SK, Singh R. Liposomes based nasal delivery system of nifedipine: Development and characterization. J Int Pharmaceutics 1995;118(1):23–30. doi:10.1016/0378-5173(94)00296-H.
[39] Salade L, Wauthoz N, Vermeersch M, Amighi K, Goole J. Chitosan-coated liposome dry-powder formulations loaded with ghrelin for nose-to-brain delivery. Eur J Pharm Biopharm 2018;129:257–266. doi:10.1016/j.ejpb.2018.06.011, PMID:29902517.
[40] Narayan R, Singh M, Ranjan O, Nayak Y, Garg S, Shavi GV, *et al*. Development of risperidone liposomes for brain targeting through intranasal route. Life Sci 2016;163:38–45. doi:10.1016/j.lfs.2016.08.033, PMID:27593571.
[41] Bassetti M, Vena A, Russo A, Peghin M. Inhaled Liposomal Antimicrobial Delivery in Lung Infections. Drugs 2020;80(13):1309–1318. doi:10.1007/s40265-020-01359-z, PMID:32691293.
[42] Wu W, Lu Y, Qi J. Oral delivery of liposomes. Ther Deliv 2015;6(11):1239–1241. doi:10.4155/tde.15.69, PMID:26584253.
[43] Pantze SF, Parmentier J, Hofhaus G, Fricker G. Matrix liposomes: A solid liposomal formulation for oral administration. Eur J Lipid Sci Technol 2014;116:1145–1154. doi:10.1002/ejlt.201300409.
[44] Hua S. Orally administered liposomal formulations for colon targeted drug delivery. Front Pharmacol 2014;5:138. doi:10.3389/fphar.2014.00138, PMID:24959147.
[45] Patel H, Shah C, Shah V, Upadhyay U. Factorial designing, development and in-vitro evaluation of liposomal gel for topical delivery of quetiapine. J AAPS 2012;7(4):290–302.
[46] Wang Y, Grainger DW. Lyophilized liposome-based parenteral drug development: Reviewing complex product design strategies and current regulatory environments. Adv Drug Deliv Rev 2019;151-152:56–71. doi:10.1016/j.addr.2019.03.003, PMID:30898571.
[47] Kumar V, Banga AK. Intradermal and follicular delivery of adapalene liposomes. Drug Dev Ind Pharm 2016;42(6):871–879. doi:10.3109/03639045.2015.1082580, PMID:27031916.
[48] Hurler J, Berg OA, Skar M, Conradi AH, Johnsen PJ, Skalko-Basnet N. Improved burns therapy: liposomes-in-hydrogel delivery system for mupirocin. J Pharm Sci 2012;101(10):3906–3915. doi:10.1002/jps.23260, PMID:22777770.
[49] Doppalapudi S, Mahira S, Khan W. Development and in vitro assessment of psoralen and resveratrol co-loaded ultradeformable liposomes for the treatment of vitiligo. J Photochem Photobiol B 2017;174:44–57. doi:10.1016/j.jphotobiol.2017.07.007, PMID:28753523.
[50] Srisuk P, Thongnopnua P, Raktanonchai U, Kanokpanont S. Physicochemical characteristics of methotrexate-entrapped oleic acid-containing deformable liposomes for in vitro transepidermal delivery targeting psoriasis treatment. Int J Pharm 2012;427(2):426–434. doi:10.1016/j.ijpharm.2012.01.045, PMID:22310459.
[51] Barros NB, Migliaccio V, Facundo VA, Ciancaglini P, Stábeli RG, Nicolete R, *et al*. Liposomal-lupane system as alternative chemotherapy against cutaneous leishmaniasis: macrophage as target cell. Exp Parasitol 2013;135(2):337–343. doi:10.1016/j.exppara.2013.07.022, PMID:23933281.
[52] Zhang X, Qi J, Lu Y, He W, Li X, Wu W. Biotinylated liposomes as potential carriers for the oral delivery of insulin. Nanomedicine 2014;10(1):167–176. doi:10.1016/j.nano.2013.07.011, PMID:23891617.
[53] Belogurov AA Jr, Stepanov AV, Smirnov IV, Melamed D, Bacon A, Mamedov AE, *et al*. Liposome-encapsulated peptides protect against experimental allergic encephalitis. FASEB J 2013;27(1):222–231. doi:10.1096/fj.12-213975, PMID:23047895.
[54] Gouveia VM, Lopes-de-Araújo J, Costa Lima SA, Nunes C, Reis S. Hyaluronic acid-conjugated pH-sensitive liposomes for targeted delivery

of prednisolone on rheumatoid arthritis therapy. Nanomedicine (Lond) 2018;13(9):1037–1049. doi:10.1016/j.jconrel.2011.12.024, PMID:29790395.
[55] Wong CY, Al-Salami H, Dass CR. Recent advancements in oral administration of insulin-loaded liposomal drug delivery systems for diabetes mellitus. Int J Pharm 2018;549(1-2):201–217. doi:10.1016/j.ijpharm.2018.07.041, PMID:30071309.
[56] Zeb A, Qureshi OS, Yu CH, Akram M, Kim HS, Kim MS, *et al*. Enhanced anti-rheumatic activity of methotrexate-entrapped ultradeformable liposomal gel in adjuvant-induced arthritis rat model. Int J Pharm 2017;525(1):92–100. doi:10.1016/j.ijpharm.2017.04.032, PMID:28428089.
[57] Gibbons A, Padilla-Carlin D, Kelly C, Hickey AJ, Taggart C, McElvaney NG, *et al*. The effect of liposome encapsulation on the pharmacokinetics of recombinant secretory leukocyte protease inhibitor (rSLPI) therapy after local delivery to a guinea pig asthma model. Pharm Res 2011;28(9):2233–2245. doi:10.1007/s11095-011-0454-1, PMID:21647791.
[58] Li Y, Su T, Zhang Y, Huang X, Li J, Li C. Liposomal co-delivery of daptomycin and clarithromycin at an optimized ratio for treatment of methicillin-resistant Staphylococcus aureus infection. Drug Deliv 2015;22(5):627–637. doi:10.3109/10717544.2014.880756, PMID:24471983.
[59] He J, Abdelraouf K, Ledesma KR, Chow DS, Tam VH. Pharmacokinetics and efficacy of liposomal polymyxin B in a murine pneumonia model. Int J Antimicrob Agents 2013;42(6):559–564. doi:10.1016/j.ijantimicag.2013.07.009, PMID:24016799.
[60] Patil JS, Devi VK, Devi K, Sarasija S. A novel approach for lung delivery of rifampicin-loaded liposomes in dry powder form for the treatment of tuberculosis. Lung India 2015;32(4):331–338. doi:10.4103/0970-2113.159559, PMID:26180381.
[61] Papadia K, Markoutsa E, Mourtas S, Giannou AD, La Ferla B, Nicotra F, *et al*. Multifunctional LUV liposomes decorated for BBB and amyloid targeting. A. In vitro proof-of-concept. Eur J Pharm Sci 2017;101:140–148. doi:10.1016/j.ejps.2017.02.019, PMID:28193538.
[62] Xiang Y, Wu Q, Liang L, Wang X, Wang J, Zhang X, *et al*. Chlorotoxin-modified stealth liposomes encapsulating levodopa for the targeting delivery against Parkinson's disease in the MPTP-induced mice model. J Drug Target 2012;20(1):67–75. doi:10.3109/1061186X.2011.595490, PMID:22149216.
[63] Chen ZL, Huang M, Wang XR, Fu J, Han M, Shen YQ, *et al*. Transferrin-modified liposome promotes α-mangostin to penetrate the blood-brain barrier. Nanomedicine 2016;12(2):421–430. doi:10.1016/j.nano.2015.10.02, PMID:26711963.
[64] Moreno LC, Cavalcanti IM, Satyal P, Santos-Magalhães NS, Rolim HM, Freitas RM. Acute toxicity and anticonvulsant activity of liposomes containing nimodipine on pilocarpine-induced seizures in mice. Neurosci Lett 2015;585:38–42. doi:10.1016/j.neulet.2014.11.025, PMID:25445375.
[65] Cao X, Hou D, Wang L, Li S, Sun S, Ping Q, *et al*. Effects and molecular mechanism of chitosan-coated levodopa nanoliposomes on behavior of dyskinesia rats. Biol Res 2016;49(1):32. doi:10.1186/s40659-016-0093-4, PMID:27378167.
[66] Kempwade AA, Taranalli AD, Hiremath RD, Joshi SA. Formulation and Evaluation of Flexible Liposome Embedded *In Situ* Thermoreversible Intranasal Gel of Rizatriptan Benzoate. Indian J Pharm Sci 2022;84(4):863–873. doi:10.36468/pharmaceutical-sciences.981.
[67] Fukuta T, Ishii T, Asai T, Sato A, Kikuchi T, Shimizu K, *et al*. Treatment of stroke with liposomal neuroprotective agents under cerebral ischemia conditions. Eur J Pharm Biopharm 2015;97(Pt A):1–7. doi:10.1016/j.ejpb.2015.09.020, PMID:26455340.
[68] Benne N, van Duijn J, Lozano Vigario F, Leboux RJT, van Veelen P, Kuiper J, *et al*. Anionic 1,2-distearoyl-sn-glycero-3-phosphoglycerol (DSPG) liposomes induce antigen-specific regulatory T cells and prevent atherosclerosis in mice. J Control Release 2018;291:135–146. doi:10.1016/j.jconrel.2018.10.028, PMID:30365993.
[69] Li X, Xiao H, Lin C, Sun W, Wu T, Wang J, *et al*. Synergistic effects of liposomes encapsulating atorvastatin calcium and curcumin and targeting dysfunctional endothelial cells in reducing atherosclerosis. Int J Nanomedicine 2019;14:649–665. doi:10.2147/IJN.S189819, PMID:30697048.
[70] Qu Y, Tang J, Liu L, Song L, Chen S, Gao Y. α-Tocopherol liposome loaded chitosan hydrogel to suppress oxidative stress injury in cardiomyocytes. Int J Biol Macromol 2019;125:1192–1202. doi:10.1016/j.ijbiomac.2018.09.092, PMID:30227207.
[71] Haeri A, Sadeghian S, Rabbani S, Anvari MS, Erfan M, Dadashzadeh S. PEGylated estradiol benzoate liposomes as a potential local vascular delivery system for treatment of restenosis. J Microencapsul 2012;29(1):83–94. doi:10.3109/02652048.2011.630107, PMID:22047547.
[72] Zare Kazemabadi F, Heydarinasab A, Akbarzadeh A, Ardjmand M. Preparation, characterization and *in vitro* evaluation of PEGylated nanoliposomal containing etoposide on lung cancer. Artif Cells Nanomed Biotechnol 2019;47(1):3222–3230. doi:10.1080/21691401.2019.1646265, PMID:31373225.
[73] Elbayoumi TA, Torchilin VP. Enhanced cytotoxicity of monoclonal anticancer antibody 2C5-modified doxorubicin-loaded PEGylated liposomes against various tumor cell lines. Eur J Pharm Sci 2007;32(3):159–168. doi:10.1016/j.ejps.2007.05.113, PMID:17707615.
[74] Yao C, Wang P, Li X, Hu X, Hou J, Wang L, *et al*. Near-Infrared-Triggered Azobenzene-Liposome/Upconversion Nanoparticle Hybrid Vesicles for Remotely Controlled Drug Delivery to Overcome Cancer Multidrug Resistance. Adv Mater 2016;28(42):9341–9348. doi:10.1002/adma.201503799, PMID:27578301.
[75] Vartak R, Patil SM, Saraswat A, Patki M, Kunda NK, Patel K. Aerosolized nanoliposomal carrier of remdesivir: an effective alternative for COVID-19 treatment in vitro. Nanomedicine (Lond) 2021;16(14):1187–1202. doi:10.2217/nnm-2020-0475, PMID:33982600.
[76] Shah VH, Jobanputra A. Enhanced Ungual Permeation of Terbinafine HCl Delivered Through Liposome-Loaded Nail Lacquer Formulation Optimized by QbD Approach. AAPS PharmSciTech 2018;19(1):213–224. doi:10.1208/s12249-017-0831-0, PMID:28681334.
[77] Spellberg B, Walsh TJ, Kontoyiannis DP, Edwards J Jr, Ibrahim AS. Recent advances in the management of mucormycosis: from bench to bedside. Clin Infect Dis 2009;48(12):1743–1751. doi:10.1086/599105, PMID:19435437.
[78] De Leo V, Milano F, Agostiano A, Catucci L. Recent Advancements in Polymer/Liposome Assembly for Drug Delivery: From Surface Modifications to Hybrid Vesicles. Polymers (Basel) 2021;13(7):1027. doi:10.3390/polym13071027, PMID:33810273.
[79] Ning B, Huang Z, Youngquist BM, Scott JW, Niu A, Bojanowski CM, *et al*. Liposome-mediated detection of SARS-CoV-2 RNA-positive extracellular vesicles in plasma. Nat Nanotechnol 2021;16(9):1039–1044. doi:10.1038/s41565-021-00939-8, PMID:34294909.
[80] Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and Challenges of Liposome Assisted Drug Delivery. Front Pharmacol 2015;6:286. doi:10.3389/fphar.2015.00286, PMID:26648870.
[81] Levchenko TS, Hartner WC, Torchilin VP. Liposomes in diagnosis and treatment of cardiovascular disorders. Methodist Debakey Cardiovasc J 2012;8(1):36–41. doi:10.14797/mdcj-8-1-36, PMID:22891109.
[82] Lin C, Gao H, Ouyang L. Advance cardiac nanomedicine by targeting the pathophysiological characteristics of heart failure. J Control Release 2021;337:494–504. doi:10.1016/j.jconrel.2021.08.002, PMID:34358590.