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REVIEW ARTICLE

Advances in Liposome Technology: An Intensive Review of Formulation, Therapeutic Applications, and Challenges

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Abstract: ***Introduction:*** Liposomes are versatile drug delivery vehicles due to their nanoscale lipid bilayer vesicles, capable of encapsulating both hydrophilic and hydrophobic substances. They have shown promise in vaccine development, gene therapy, cancer treatment, and targeted drug delivery. However, their clinical applicability is limited due to factors like drug stability, manufacturing constraints, regulatory challenges, and immune responses. This study explores liposome formulations by focusing on enhanced stability, robustness, and drug-loading efficiency. It also discusses therapeutic implementation challenges.

Methods: A systematic literature review was conducted using specific keywords and Boolean operators across databases, such as Web of Science, PubMed, and Scopus. Non-peer-reviewed articles, conference abstracts, and studies with poor methodology were excluded.

Results: This review highlights advances in liposome formulation that boost therapeutic performance, enhance stability, and improve drug loading. Despite their promise, clinical application depends on overcoming issues like manufacturing complexity, regulatory constraints, and immune reaction limitations.

Discussion: Liposomes enable efficient encapsulation and targeted delivery for both hydrophilic and hydrophobic drugs, enhancing therapeutic efficacy. Their biocompatibility makes them effective in cancer therapy, vaccine transport, and gene delivery. Nevertheless, further research is needed to improve production processes and ensure long-term safety for regulatory approval and commercial scalability.

Conclusion: Liposomes hold strong potential for medical use and drug delivery. To achieve broader clinical adoption, challenges in formulation and regulation must be addressed. This review highlights recent innovations and strategies to optimize liposome-based therapeutics.

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1. INTRODUCTION

A liposome is a tiny, spherical vesicle composed of one or more phospholipid bilayers, resembling the structure of a cell membrane. The term liposome originates from the Greek words lipos and soma, meaning fat and body, respectively. Liposomes can be classified into different types based on various characteristics; for example, they can be categorized by structural composition as multilamellar vesicles (MLVs) and unilamellar vesicles (ULVs), which are further divided into small unilamellar vesicles (SUVs) and large unilamellar vesicles (LUVs). Additionally, categorization can be based on preparation method, composition, and application [1, 2]. The versatility of liposomes allows encapsulation of both hydrophilic and hydrophobic drugs, enhancing targeted delivery and bioavailability. Moreover, liposomes can be surface-modified to evade immune detection, prolonging their circulation time. This advanced delivery system not only boosts therapeutic efficacy but also minimizes side effects by limiting drug exposure to healthy tissues [3].

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Dr. Alec D. Bangham FRS, a British hematologist at Cambridge's Babraham Institute, first described liposomes in 1961. They were discovered when Dr. Bangham and his colleague R. W. Horne used negative staining on dried phospholipids to test a new electron microscope. The resulting images provided the first clear evidence of the bilayer lipid structure of the cell membrane, resembling the plasmalemma [4].

Liposomal formulations for both small-molecule therapies and large-molecule biologics have been widely adopted by academic and corporate research groups [5]. Liposomal drug delivery systems have yielded promising therapeutic outcomes [6]. A broad spectrum of drugs, varying in size, composition, and characteristics, such as peptide hormones, proteins, chelating agents, enzymes, vaccines, anticancer and antimicrobial agents, and genetic materials, have been incorporated into the aqueous or lipid phases of liposomes to ensure targeted *in vivo* delivery. As mentioned earlier, liposomes can be characterized based on their lamellarity (unilamellar or multilamellar vesicles), size (small [~100 nm], intermediate [100–250 nm], large [>250 nm]), and surface charge (anionic, cationic, or neutral) [7].

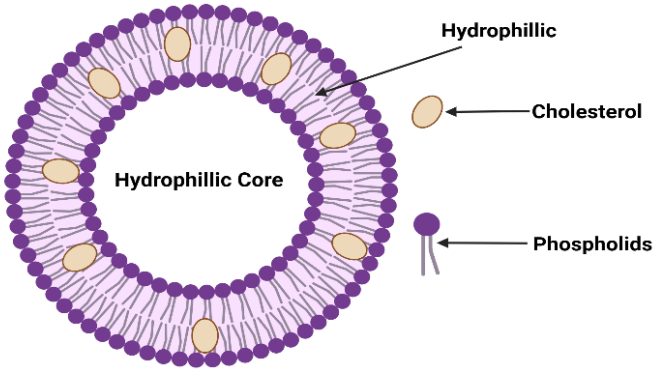

Fig. (1). A diagrammatic representation of liposomes. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Fig. (**1**) shows the physicochemical properties of liposomes, which affect their removal from the body and systemic circulation. These properties include size, water-repelling (hydrophobicity), water-attracting (hydrophilicity), and net surface charge. Surface water-repelling and size (> 200 nm) enhance opsonization and reticuloendothelial system (RES) absorption. Liposomes have various advantages as a drug delivery system, including biocompatibility, self-assembly capability, the potential to transport large drug payloads, and a wide variety of physicochemical and biophysical properties that can be changed to influence their biological features [8]. Liposomes have long received increased scientific attention in pharmaceuticals. Many large-scale techniques have been described in the recent past. They include the age-old extrusion method, electro formation, freeze-drying, hydration or swelling double emulsions (water-in-oil-in-water, or W/O/W), and bubbling [9]. Further advancements in liposome technology have paved the way for numerous research studies and have led to various products entering clinical trials [9]. Doxil®, the very first product of Gabizon and Barenholz, helped to develop many anticancer formulations, such as DaunoXome®, Depocyt®, Myocet®, Mepact®, Marqibo®, and OnivydeTM. Liposomal studies are not only effective in the anticancer therapy, but they are also highly beneficial for developing antifungal drugs, such as Abelcet®, Ambisome®, and Amphotec®, pain relievers, such as DepoDurTM and Exparel®, and oral antiviral medications like Epaxal® and Inflexal® V. This clearly demonstrates that the development of liposome technology is expanding in multiple directions, as evidenced by the increasing number of liposomal formulations used in various clinical studies. To achieve substantial improvements for a broad range of patients, it is essential to continue conducting clinical studies on liposomal formulations across all possible areas [9]. Increasing clinical trials have shown that the applications of liposomes are expanding. Current formulations are expected to help a variety of patient categories. Moreover, integrating liposomes with biopolymers improves both *in vitro* and *in vivo* results. Second-generation biopolymer-liposome hybrids, which are biocompatible and biodegradable, have been utilized in food, cosmetic, and medicinal applications [7]. This study aims to review advances in liposome technology related to liposomal development and pharmaceutical applications. It also highlights some future nanotechnological approaches.

2. METHODOLOGY

This study employed a systematic approach to ensure a comprehensive and unbiased selection of relevant publications. A thorough literature search was conducted using predefined keywords and Boolean operators across multiple databases, including Web of Science, PubMed, and Scopus. The primary goal of the search strategy was to identify studies on advances in liposome technology by focusing on their formulation, therapeutic applications, and challenges. Non-peer-reviewed sources, conference abstracts, and studies with insufficient methodological detail were excluded, while peer-reviewed English-language publications containing empirical data or systematic analyses met the inclusion criteria. A full-text review was performed on eligible articles after screening abstracts and titles. The selected publications were analysed for key trends, notable findings, and research gaps to provide a critical review of the existing body of literature.

3. LIPOSOME ARCHITECTURE

Liposomes are highly versatile structures that can be produced through various methods, resulting in significant variation in their size, shape, and surface properties. They are classified based on size and the number of layers, such as unilamellar and multilamellar vesicles. The size and charge of liposomes depend on the preparation method and lipid composition. Generally, small unilamellar vesicles (SUVs) range from 0.02 to 5.0 µm, while multilamellar vesicles (MLVs) range from 0.1 to 0.05 µm. In contrast, large unilamellar vesicles (LUVs) typically measure at least 0.06 µm in

diameter. Lamellarity, or the number of layers that make up the vesicle shell, size distribution, and captured volume are some of the characteristics that define lipid vesicles [10]. Kinetic investigations of liposome-complement interactions have been carried out to determine the affinity (Km) and capacity (μmax) of the complement system for releasing encapsulated carboxyfluorescein [10]. Given the diversity of liposomal preparations, precise estimation of size-frequency distribution is essential. Light-scattering methods, while commonly used, rely on algorithms that may yield misleading results. For example, Yamada *et al*. examined carboxyfluorescein release from liposomes of three different diameters (800, 400, and 200 nm) at varying concentrations (1 to 1,000 nmol/mL). At low concentrations (1–10 nmol/mL), small liposomes (200 nm) released carboxyfluorescein at a rate comparable to medium (400 nm) and large (800 nm) liposomes, which is approximately 35%. However, light-scattering techniques are often unable to distinguish between a large particle and a flocculated mass of smaller particles. Importantly, any micron-sized particles in such aggregates may need to be removed prior to analysis [10]. Classification of liposomes is based on structure, preparing process, application and composition, standard liposomes, and liposome specialties.

3.1. Classification Based on Structure

Table **1** presents the size and number of lipid bilayers of different types of vesicles. Liposomes can be subdivided into main categories based on the structural parameters mentioned in Table **1**.

Additionally, unilamellar vesicles (ULVs) can be classified into four size-based classes: giant unilamellar vesicles (GUVs), small unilamellar vesicles (SUVs), large unilamellar vesicles (LUVs), and medium unilamellar vesicles (MULs) (Fig. **2**). The circulatory half-life of these liposome vesicles is significantly influenced by their size [11, 12]. Liposomes can interact with cellular membranes by a variety of mechanisms, including phagocytosis, local fusion (attachment), selective (ligand-mediated) or nonspecific endocytosis, and membrane uptake. Numerous factors influence liposome–cell interactions, including the liposome's composition, size, surface charge, presence of surface-targeting ligands, and the surrounding biological environment [13].

4. LIPOSOMES COMPOSITION

Liposomes are primarily composed of phospholipids, which can be categorized into two main types: glycerophospholipids and sphingomyelins. Glycerophospholipids consist of a hydrophilic head group and a hydrophobic side chain. Variations in the head group result in a variety of glycerophospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), phosphatidylglycerol (PG), and cardiolipin (CL)[3]. Additionally, differences in the length of the nonpolar moieties lead to distinct glycerophospholipids, such as dimyristoyl, dipalmitoyl, and distearoyl PC. The type of bond (ether or ester) between glycerol and aliphatic chains also contributes to the diversity of glycerophospholipids [14]. Lipids share a common structural feature: a hydrophilic head group and a hydrocarbon tail that is hydrophobic. Depending on whether the head group is negatively or positively charged, or zwitterionic, that is, composed of both positive and negative charges, the molecule can be overall neutral. The composition of liposome-associated lipids includes natural lipids, synthetic lipids, sterols, and surfactants, each contributing to the properties and functionality of liposomes [13].

4.1. Liposome-Related Lipids and Phospholipids

Liposomes are multilayered or spherical vesicles, formed in aqueous solutions as diacyl-chain phospholipids self-assemble into lipid bilayers. These bilayers are made up of phospholipids with hydrophobic tails and hydrophilic heads, creating an amphiphilic configuration. Both synthetic and natural phospholipids can be used to create liposomes. Lipid content has a major impact on essential liposome characteristics, including electrostatic charge, stability, fluidity, stiffness, and particle size [13]. Liposomes containing natural unsaturated phosphatidylcholine, similar to those found in eggs or soybeans, have low stability but good permeability. Saturated phospholipid-based liposomes, including dipalmitoyl phosphatidylcholine, have a hard and almost imper-

Table 1. Different types of liposomes.

Vesicles	Diameter Size	No of Lipid Bilayers
Multilamellar liposomes/vesicles (MLVs)	50 nm to 150 nm	Multi-compartmental structure
Oligolamellar vesicles (OLVs)	0.1-1 micrometer	Approx. 5
Unilamellar vesicles (ULVs)	All size ranges	One
Large unilamellar vesicles (LUVs)	More than 100 nm	One
Giant unilamellar vesicles (GUVs)	More than 1 micrometer	One
Multilamellar vesicles (MLVs)	More than 0.5	5-25
Multi-vesicular vesicles (MVVs)	More than 1 micrometer	Multi-compartmental structure
Small unilamellar vesicles (SUVs)	20-100 nm	One
Medium unilamellar vesicles (MUVs)	More than100 nm	One

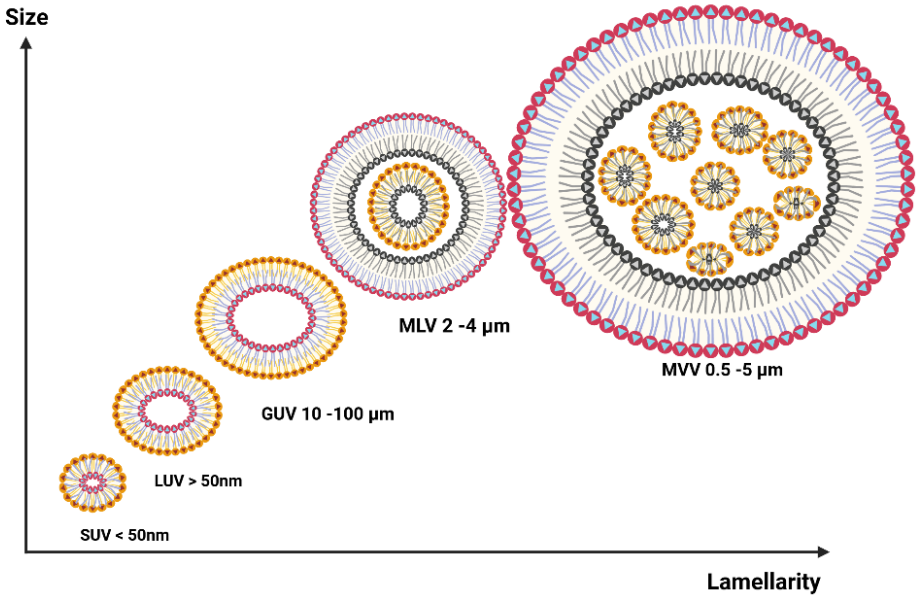

Fig. (2). Diagrammatic representation of liposomes and classification by vesicle number. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

meable bilayer structure. The hydrophilic groups of the lipids can be zwitterionic, positively charged, or negatively charged, offering stability through electrostatic repulsion. The hydrophobic group, on the other hand, differs in terms of saturation, uniformity, and acyl chain length [13]. Lipids in general have a similar structural framework that consists of a hydrophilic head group and a hydrophobic hydrocarbon tail. The head group's charge can be negative, positive, or zwitterionic, contributing to liposome structural and functional diversity [13].

4.2. Natural Lipids

Natural phospholipids are mostly derived from egg yolks and soybeans. Phospholipids, such as phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidic acid (PA), are categorized based on their polar head groups. Due to the unsaturated nature of their hydrocarbon chains, compared to manufactured phospholipids, natural phospholipids are less stable during liposome synthesis. Natural phospholipids include fatty acids, including hexadecanoic acid (palmitic acid, $H_3C–(CH_2)_{14}–COOH$), heptadecanoic acid (margaric acid, $H_3C–(CH_2)_{15}–COOH$), and oleic acid (9Z-octadecenoic acid), which is typically present in egg yolk lecithin [13]. Phosphatidyl ethanolamine (PE), for example, is frequently conjugated with polyethylene glycol using its amine group. Charged liposomes are stable due to electrostatic repulsion, and variables in the hydrophobic tails, such as acyl chain length, symmetry, and saturation, further influence their characteristics. Frequently, phosphate, glycerol, or sphingosine groups make up the lipid backbone. These properties determine essential liposome behaviours, such as bilayer formation, lipid packing, pH responsiveness, stability, and drug encapsulation and release [13].

Approximately 92% of the total fatty acid content of egg phospholipids and PCs is composed of fatty acids, including arachidonic acid (C20:4), oleic acid (C18:1), linoleic acid (C18:2), palmitic acid (C16:0), and stearic acid (C18:0). Unsaturated fatty acids, including docosahexaenoic acid (C22:6, n-3) and arachidonic acid (C20:4, n-6), are prevalent in egg phospholipids. Around 40% of egg PCs are composed of 1-palmitoyl-2-oleoylphosphatidylcholine. Similarly, soybean-derived phospholipids predominantly consist of oleic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), palmitic acid (C16:0), and stearic acid (C18:0), which together make up roughly 95% of the fatty acid profile [13].

4.3. Synthetic Lipids

Commercially produced synthetic lipids, which are not found in nature or generated from living things, are commonly used in therapeutic liposomes. While these lipids do not naturally exist in the body, they share biochemical and structural similarities with natural lipids and exhibit a high degree of biocompatibility. Synthetic phospholipids are primarily based on saturated fatty acids, such as stearic and palmitic acids [14]. These lipids are created through certain chemical changes to the polar and non-polar parts of natural phospholipids, which allow for the creation of a wide variety of distinct and classified phospholipids [13].

Furthermore, mixed fatty acids, unsaturated fatty acids in both hydrocarbon chains, or unsaturated fatty acids in just one chain can all be used to create synthetic phospholipids. Common synthetic lipids consist of phosphatidylcholines, phosphatidylethanolamines, and phosphatidylglycerols, including dioleoyl phosphatidylglycerol (DOPG), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). Due to their greater purity, broad commercial availability, adaptable

chemical functionality, and affordability, synthetic lipids are frequently chosen over natural lipids [13].

4.4. Sterols

Nearly all living organisms contain sterols, a class of natural lipids. Three subtypes are distinguished among them: mycosterols in microbes, phytosterols in plants, and zoosterols in animals. The cholesterol, also known as zoosterol, is the most common steroid utilized in liposome manufacturing, accounting for less than 30% of total lipids. Cholesterol improves liposome stiffness and stability by incorporating into the lipid bilayers. The effects of cholesterol and β-sitosterol on the properties of liposome membranes revealed that both steroids increase absolute zeta potential and decrease membrane fluidity, alter particle size, and lower the enthalpy and DPPC phase transition temperature (Tm). These findings highlight the significance of sterols in modifying liposomal characteristics [13].

Cholesterol, a vital part of mammalian cell membranes, is an endogenous amphiphilic zoosterol. It is mostly present in lipid rafts, where it keeps the membrane intact and regulates lipid raft activity. The addition of cholesterol to liposomal formulations increases stability *in vivo* and reduces lipid bilayer leakage. Liposomes containing 20-50 mol% cholesterol, for example, demonstrated lower encapsulation efficiency but much higher stability *in vivo* compared to cholesterol-free controls. Cholesterol-rich liposomes can survive in the bloodstream for more than six hours, whereas cholesterol-free liposomes disintegrate in minutes [13].

4.5. Surfactants

Surfactants, also known as surface-active agents or edge activators, are amphipathic chemicals made up of two primary moieties: a polar hydrophilic component and a non-polar lipophilic portion. Surfactants exist as monomers in aqueous solutions in trace amounts and preferentially adsorb at interfacial surfaces. This adsorption displaces surface molecules, weakens intermolecular interactions, and reduces surface tension. Furthermore, surfactant molecules self-assemble into micelles when their concentration reaches the critical micelle concentration (CMC), which is a specific concentration threshold. According to studies, increasing the temperature of the solution reduces the CMC value by breaking down hydrogen bonds between the hydrophilic groups of surfactants and water molecules [15].

Surfactants play a crucial role in liposome formulations, altering the ability of liposomes to encapsulate and release by lowering surface tension between immiscible phases. Surfactants break down the lipid bilayer of liposomal nanoparticles by functioning as single-chain amphiphiles, leading to the formation of additional nanovesicles. These surfactant-containing liposomes have been widely utilized in drug delivery to enhance the skin penetration of encapsulated therapeutic compounds. For example, ultra-deformable liposomes, also known as transferosomes, have shown great promise in transdermal drug delivery. The edge activator, a key component in these systems, enhances the flexibility of lipid bilayers, enabling vesicles to undergo rapid shape changes in response to osmotic pressure with minimal energy input [13, 15].

This deformability enhances transdermal drug permeation, making these nanovesicles particularly suitable for topical administration of antihypertensive medications. Edge activators have also proven effective in enhancing dermal penetration for anticancer, antifungal, and other transdermal applications. In some cases, therapeutic efficacy can be increased by taking advantage of the edge activators' charge. For example, negatively charged substances like DNA can develop electrostatic interactions with sodium cholate, which has a positive zeta potential. Furthermore, the therapeutic effectiveness of liposomal drug and gene delivery might be influenced by the kind and concentration of surfactant systems. Surfactants are typically classified based on their molecular weight or hydrophilic-lipophilic balance. They are broadly categorized into two groups: (A) low molecular weight surfactants and (B) polymeric surfactants [13].

5. SIZING OF LIPOSOMES

Liposome size is an important factor in determining their fate and therapeutic uses. The physical integrity and stability of the lipid bilayer structure are essential for efficient medication delivery. As a result, the liposome synthesis technique must be consistent, reproducible, and capable of producing particle size distributions within a specified range. Lipid-based formulations are intended to function as site-specific drug delivery vesicles. They are frequently removed by Kupffer cells in the liver and macrophages, necessitating techniques to escape detection by the reticuloendothelial system (RES) while ensuring efficient transport of liposome-incorporated compounds to the target tissue, organ, or tumour. Liposomes are commonly manufactured by consecutive low-pressure extrusion through a polycarbonate membrane (PCM) with a 0.27 micrometre hole size. This membrane extrusion mechanism is essential for transforming large unilamellar vesicles (LUVs), and extrusion procedures convert multilamellar vesicles (MLVs) into large unilamellar vesicles (LUVETs). Other methods for sizing liposomes include gel chromatography and sonication. Gel chromatography is typically used to measure the size of liposomes and can also separate encapsulating components as needed. Sonication, another popular approach, has various drawbacks. The elimination of oxygen is difficult, raising the risk of lipid peroxidation. Titanium probes used for sonication may lose metal particles, resulting in contamination. Sonication can produce aerosols, making it unsuitable for some compounds [16, 17].

6. STABILITY, ZETA POTENTIAL, AND SIZE

The stability of liposomes is a crucial aspect in determining efficacy and usability in medicinal applications. Monitoring the liposomes at different intervals (days, weeks, or months) and analyzing factors like drug leakage and nanoparticle size are common methods for determining a formu-

lation's durability. The physicochemical properties of liposome formulation may be jeopardised over time by undesired changes, such as particle aggregation and lipid membrane disintegration. For liposomes, dynamic light scattering (DLS) and phase analysis light scattering (PALS) are typically used to determine particle diameter and surface charge, respectively. These measures are essential for forecasting the stability and behavior of liposomes. For example, liposomes with a neutral surface charge have a tendency to agglomerate, which makes them unstable and unsuitable for drug delivery applications [13].

7. VESICLES PREPARATION

7.1. Multilamellar Vesicle Preparation

The most fundamental method for synthesizing liposomes involves the formation of multilamellar vesicles (MLVs). Using this technique, the lipids are dissolved in organic solvents and then dried to form the resulting lipid mixture. When used at a molar ratio of 0.9:1.0:0.1, the typical lipids include phosphatidylglycerol, cholesterol, and egg lecithin. The solvent is either chloroform or a normal 2:1 mixture of chloroform and methanol. To guarantee a uniform distribution, the lipids are first dissolved individually before being combined with the organic solvent in the proper amounts. After that, a thin lipid layer is created in the test tube using a nitrogen stream. To eliminate any residual organic solvent, the lipid film is vacuum-dried for at least four to six hours [9].

7.2. Unilamellar Vesicle Preparation

Unilamellar vesicles are the most common type of liposomes. Their structure ensures that the chemicals encapsulated within them are evenly distributed inside a single, interior aqueous compartment. These vesicles can be synthesized through various methods, including ultrasonication, extrusion through polycarbonate filters, freeze-thawing, ethanol injection, the detergent method, and the creation of sterile large unilamellar vesicles (LUVs). Bhatia *et al*. (2015) utilized a blend of distinct small unilamellar vesicles (SUVs) to create ternary giant unilamellar vesicles (GUVs) with consistent properties [9].

7.3. Giant Unilamellar Liposomes Preparation

There are several methods for creating giant liposomes using only purified water, non-electrolytes, or zwitterions. The presence of ions, which impart a net charge, enhances the attraction between membranes and helps prevent the separation of membrane sheets during the rehydration and swelling process. Recently, researchers have demonstrated the production of giant liposomes using physiological-strength buffers. These systems can be created through various techniques, including electroformation, rapid preparation of large liposomes, the use of physiological buffers for creating giant unilamellar vesicles, and the osmotic shock method [9].

8. FORMULATION METHODS

Advanced methods of liposome formulation generally follow four main stages. The process begins with drying hydrophobic lipids from natural solvents to form a thin lipid film. This is followed by dispersing the dried lipids in an aqueous phase, which leads to the formation of liposomes. The next stage involves purifying the resulting liposomes to remove any unencapsulated materials or byproducts. Finally, the completed formulation is evaluated for key parameters, such as particle size, surface charge, encapsulation efficiency, and stability. Liposomes can be produced using various methods, and their nomenclature is often determined by their preparation technique, physical characteristics, or intended function [18]. Liposomes are prepared using techniques [19], such as passive loading techniques, active loading techniques, etc.

Methods for preparing liposomes can be broadly divided into three categories (Table **2**): detergent removal, solvent dispersion, and mechanical dispersion.

8.1. Mechanical Dispersion Method

8.1.1. Lipid Film Hydration by Handshaking

Lipid film hydration by handshaking is a mechanical dispersion technique that involves dissolving lipids and hydrophobic medications in an organic solvent, evaporating the resulting thin lipid film, and then hydrating the resulting multilamellar vesicles (MLVs). In this case, the lipids are dissolved in chloroform. A thin lipid layer is then formed as the organic solvent gradually evaporates at lower pressure. In order to hydrate the lipid layer, phosphate buffer (pH 7.4) is added to one side of the flask. An aqueous solution containing the drug is then introduced to the opposite side of the flask. The flask is slowly returned to an upright orientation, allowing the aqueous medium to flow gently over the lipid film. The flask is left to stand for 2 hours at 37°C to allow complete swelling of the lipid film. After swelling, vesicles are harvested by gently swirling the flask, resulting in a milky white suspension (Fig. **3**). The formulations are then centrifuged to separate the liposomes. Different batches of liposomes are prepared to identify the optimal formulation [18].

8.1.2. Sonication

Sonication is one of the most widely employed procedures for preparing small unilamellar vesicles (SUVs). Multilamellar vesicles (MLVs) are typically sonicated using either a bath sonicator or a probe sonicator in a passive atmosphere. However, this approach has several disadvantages, including small internal volume, low encapsulation efficiency, phospholipid degradation, and the removal of large molecules. Additionally, metallic interference from the probe tip and the coexistence of MLVs alongside SUVs can complicate the process. There are two main sonication methods (Fig. **4**) [18], which are as follows:

Table 2. Methods of liposome preparation.

Mechanical dispersion method	✓ Lipid film hydration by handshaking ✓ Non-handshaking or freeze drying ✓ Micro-emulsification ✓ Sonication ✓ French-pressure cell ✓ Membrane extrusion ✓ Dried reconstituted vesicles ✓ Freeze-thawed liposomes
Solvent dispersion method	✓ Ethanol injection ✓ Ether injection ✓ Double emulsion vesicles ✓ Reverse-phase evaporation vesicles ✓ Stable plurilamellar vesicles
Detergent removal methods	✓ Detergent (chlorate, Aglycoside Triton X-100) Removal from mixed micelles by o Dialysis o Column chromatography o Dilution o Reconstituted Sendai virus envelopes

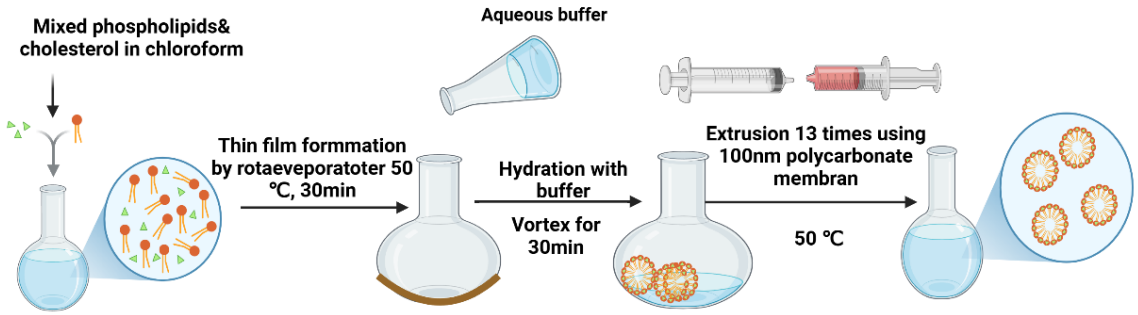

Fig. (3). Diagrammatic representation of the thin-film hydration extrusion technique. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

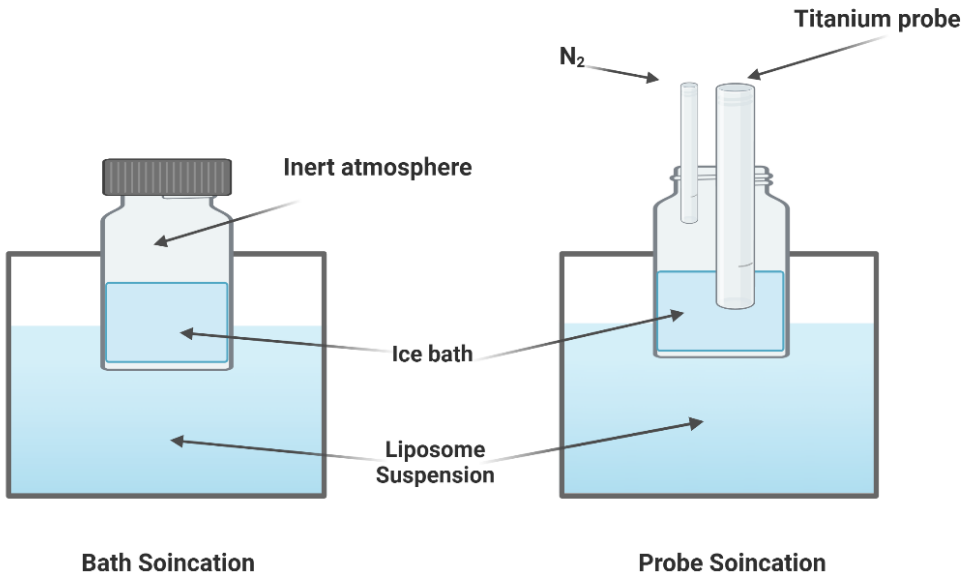

Fig. (4). Diagrammatic representation of probe sonication and bath sonication. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

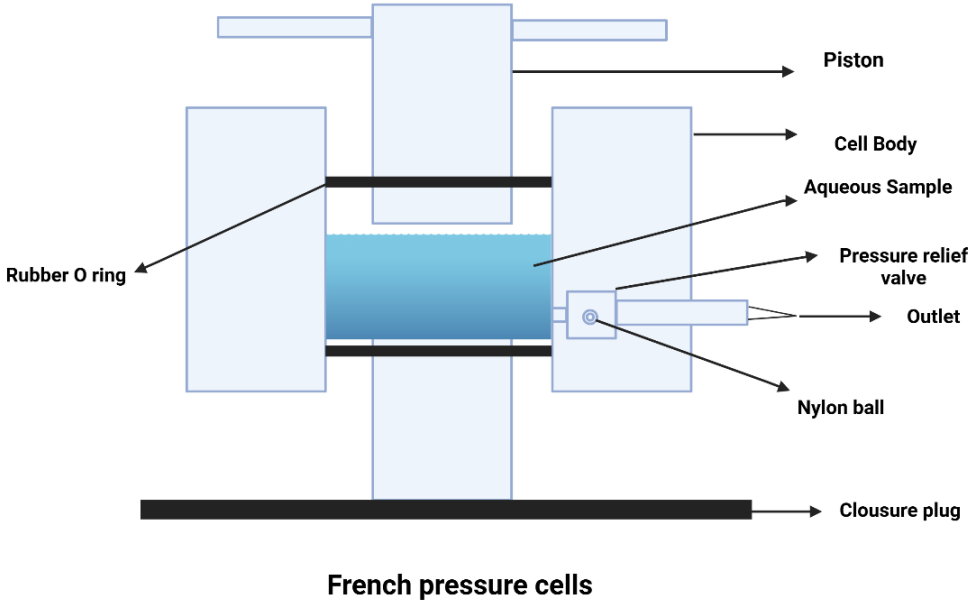

Fig. (5). Diagrammatic representation of French pressure cells. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

(a) Probe Sonication: In this method, the sonicator is directly immersed in the liposome dispersion. It requires a significant amount of energy to disperse the lipids, and the energy coupling at the tip generates localized heat. To prevent overheating, the vessels must be immersed in water or an ice bath. Prolonged sonication (up to one hour) can lead to the desertification of more than 5% of the lipids. Furthermore, the titanium probe tip may shed particles, contaminating the solution [18, 20].

(b) Bath Sonication: In this method, the cylinder containing the liposome dispersion is placed in a bath sonicator at an appropriate temperature. Bath sonication is often simpler than probe sonication, as it does not require direct dispersion of the tip into the solution. Additionally, the sample can be sonicated in a sterile vessel and stored under an inert environment, in contrast to probe sonication, which typically requires direct exposure to the sample [18, 20].

8.1.3. French Pressure Cell

French pressure cells (Fig. **5**) [21] operate by extruding MLVs (multilamellar vesicles) through a small aperture. One important advantage of this method is that it minimizes changes to the protein during the procedure, unlike sonication, which can alter the protein structure. However, the method requires careful handling of unstable conditions. Compared to sonication, the French pressure method offers several benefits. For example, it produces SUVs (small unilamellar vesicles) that are typically smaller than those obtained through sonication. Despite these advantages, the French pressure method has some drawbacks, including the difficulty of achieving high temperatures and the relatively limited volume capacity, typically around 50 ml at most [18].

8.1.4. Freeze-Thawed Liposomes

SUVs are first frozen instantly and then allowed to thaw over time. During this short sonication process, the aggregated components are dispersed into large unilamellar vesicles (LUVs). When SUVs undergo freezing and thawing, they tend to bind together, eventually forming unilamellar vesicles. However, this synthesis process is significantly prevented by raising the medium's ionic strength and phospholipid concentration. Despite these difficulties, 20% to 30% encapsulation efficiencies are typically achieved [19].

8.1.5. Micro-emulsification

To create small vesicles from a concentrated lipid suspension, a microfluidizer is used. The lipids, initially in the form of large MLVs, are added to the fluidizer. The device operates by pumping the suspension through a 5 mm screen at extremely high pressure. The fluid then passes through long microchannels, where two streams of fluid meet at right angles at very high speeds. This interaction causes the lipids to break up into smaller vesicles. The fluid, containing the vesicles, is collected and can be recycled through the system until spherically shaped vesicles are produced [18].

8.2. Solvent Dispersion Method

8.2.1. Ether Injection

With one significant exception, the ethanol injection method and the ether injection method are extremely similar. Ether, the lipid solvent in this instance, does not react with water. This makes it possible to produce more liposomes because of its greater lipid solubility than ethanol and its inability to obstruct the synthesis of liposomes. Ether is extracted from the solution in the same manner as ethanol upon

injection [22]. This approach, however, has a number of shortcomings. For example, during the injection procedure, the temperatures of the ether and water phases must differ. Furthermore, some compounds' encapsulation and the ensuing liposomes may be hampered by ether and can exhibit highly variable shapes. It is also recommended to inject the lipid suspension under vacuum and at a slower rate compared to the ethanol injection method. Despite these challenges, the ether injection approach results in liposomes with higher encapsulation efficiency. Unlike the ethanol injection method, this technique tends to produce large unilamellar vesicles (LUVs), rather than small unilamellar vesicles (SUVs) [22].

8.2.2. Ethanol Injection

This method is commonly used to generate liposomes ranging in sizes from 30 to 170 nm, typically employed for the preparation of small unilamellar vesicles (SUVs). Fig. (**6**) describes the infusion of ethanol method. The size of the liposomes is determined by factors, such as lipid concentration and injection speed. This method involves injecting lipids dissolved in an organic solvent (ethanol in this case) into the water phase while stirring, followed by the removal of the solvent. After that, the mixture is allowed to hydrate for a further fifteen minutes while being stirred. Either centrifugation through a silica gel column or rotary evaporation is used to extract the ethanol from the liposome suspension. However, this approach has a number of drawbacks. The comparatively low solubility of lipids in ethanol is one significant disadvantage, leading to low encapsulation efficiency for hydrophilic molecules. Additionally, the high ethanol content in the solution restricts the final lipid concentrations. To avoid destabilizing the liposomes and affecting lipid incorporation, the ethanol concentration should not exceed 7.5%. Despite these limitations, the ethanol injection method is particularly useful for large-scale industrial production of liposomes [22].

8.2.3. Reverse Phase Evaporation Method

The first water-in-oil emulsion is created by quickly sonicating a two-phase system made up of phospholipids dissolved in an organic solvent (such as diethyl ether, isopropyl ether, or a combination of isopropyl ether and chloroform) and an aqueous buffer. When low pressure is used to extract the organic solvents, a thick gel is formed. The remaining solvent is then removed through low-pressure, continuous rotational evaporation, resulting in the production of liposomes. This method allows for excellent encapsulation efficiency of up to 65%, even in media with low ionic strength, such as 0.01 M NaCl. Both small and large macromolecules can be successfully encapsulated using this technique. However, one major drawback is that the materials being encapsulated are exposed to short bursts of sonication and organic solvents, which could potentially affect their stability [23].

8.2.4. Detergent Removal Methods

The detergent removal methods involve dialysis and column chromatography, which are explained in the sections below [24] (Fig. **7**).

8.2.5. Dialysis

Lipids reach their critical micelle concentration (CMC) when they start to self-assemble into micelles in the presence of detergents. As the micelles are broken up by the detergent, the phospholipids gradually incorporate into the micelles and eventually coalesce to form large unilamellar vesicles (LUVs). The detergents are then removed through dialysis [16]. One of the main advantages of the detergent dialysis technique is its high reproducibility and the ability to produce liposome populations with consistent sizes. However, a

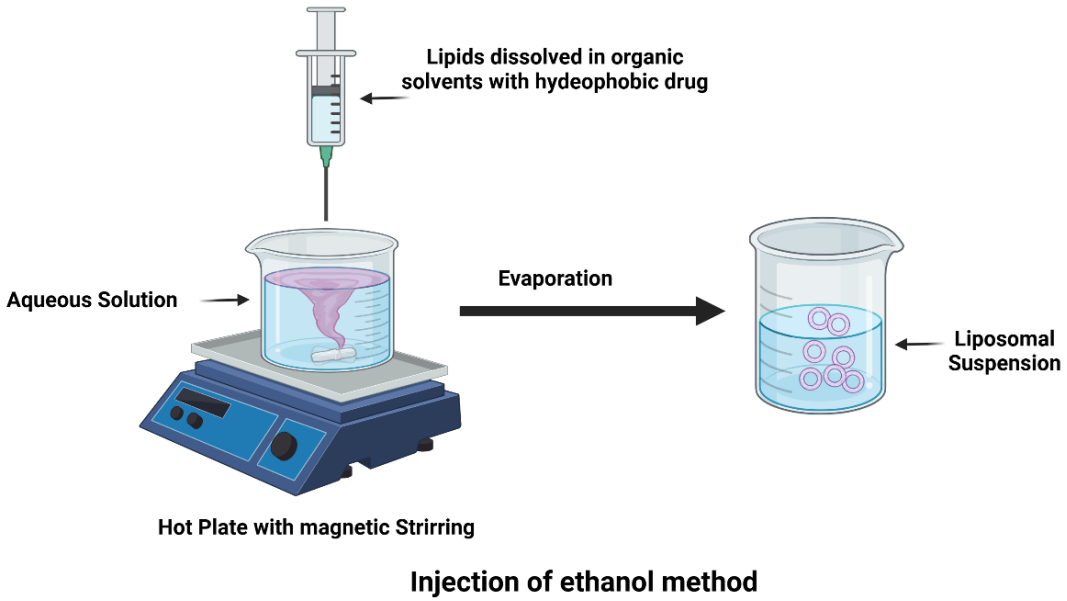

Fig. (6). A diagrammatic representation of the ethanol injection method. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

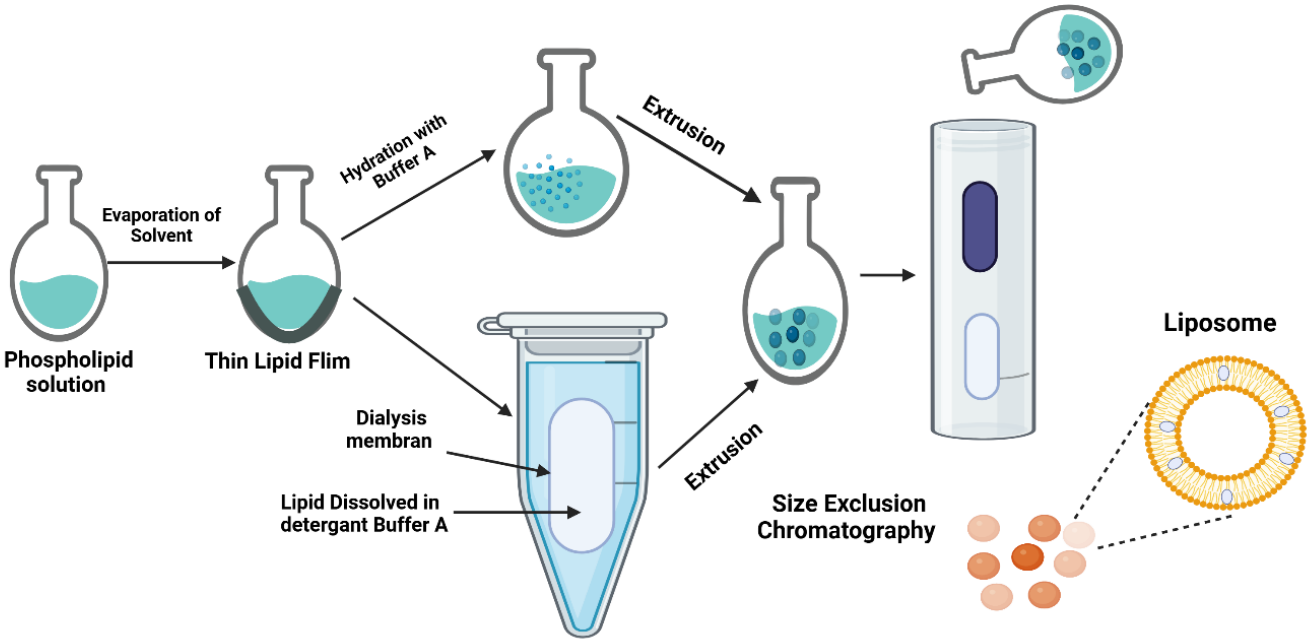

Fig. (7). A diagrammatic representation of the detergent removal method. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

significant drawback is the potential for detergent residues to remain in the liposomes [18]. A commercial version of the LIPOPREP device (Diachema AG, Switzerland) is available for detergent removal *via* dialysis. In addition, other methods for detergent removal include: (a) gel chromatography using a Sephadex G 25 column, (b) the adsorption of the detergent Triton X-100 to Bio-Beads SM -210, and (c) the use of octyl glucoside with Amberlite XAD-2 beads. Detergent adsorbers have the advantage of potentially removing detergents with low CMC that may not be fully eliminated through dialysis [19].

8.2.6. Column Chromatography

G-200 column chromatography is commonly employed to analyze the elimination of either deoxy[14C] or deoxy[3H] cholate from phosphatidylcholine, phosphatidylserine, and phosphatidylinositol. The results showed that liposomes retained a noticeably higher amount of detergent when deoxy[3H] cholate was used compared to deoxy[14C] cholate. This could be due to higher levels of impurities in the deoxy[3H] cholate or a 3H exchange between the detergent and the phospholipids. Control experiments were conducted using G-200 columns with detergent but no phospholipid. In these controls, no detergent co-eluted with the liposomes' normal empty capacity. Consequently, the detergent counts linked to the peak in void volume are most likely linked to the phospholipid vesicles themselves, rather than being caused by contamination from high molecular weight impurities, micelles, or other aggregated forms [25].

8.2.7. Dilution

Diluting an aqueous mixed micellar solution of detergent and phospholipids with buffer leads to a considerable increase in micellar size and polydispersity. A dynamic shift from polydisperse micelles to vesicles takes place as the system is further diluted past the mixed micellar phase boundary [18, 19].

9. PHARMACEUTICAL USE: DRUG DELIVERY USING LIPOSOMES

The pharmaceutical sector has seen a transformation due to liposomal drug delivery methods [26]. Since their inception, liposomes have been the subject of substantial research, and their uses in a number of fields, such as medication administration, biomolecule transfer, and gene therapy, are now well-established. The use of liposomes in pharmacology and medicine can be broadly categorized into therapeutic and diagnostic applications. They are also employed as tools or reagents in fundamental investigations into the methods of action of certain compounds, recognition processes, and cell interfaces [27]. Drugs can be encapsulated in liposomes and used to treat diseases, such as cancer [27]. In addition to their therapeutic uses, liposomes are widely recognized in numerous scientific disciplines. They serve as important models, reagents, and tools in areas, such as biophysics, colloidal chemistry, biochemistry, and biology [27]. Liposomes are considered excellent models for cell membranes and have been employed as efficient medication delivery systems, containing a variety of components, including medications, toxic substances, proteins (peptides), enzymes, antigens (antibodies), and nucleotides [28].

10. COMPARATIVE ANALYSIS OF ADVANTAGES AND LIMITATIONS OF LIPOSOMES

Liposomes offer several advantages as well as limitations across various aspects, which are outlined in Table **3**.

Table 3. Comparing the advantages and limitations of liposomes.

Aspect	Advantages	Limitations	References
Biocompatibility and biodegradability	Made from natural phospholipids, safe, and easily metabolized without toxicity.	Stability issues; prone to degradation during storage.	[59, 60]
Drug delivery	Encapsulates both hydrophilic and hydrophobic drugs, improves solubility, and protects drugs from degradation.	High production cost and complexity in large-scale manufacturing.	[59, 61]
Targeted delivery	Surface modification allows for cell/tissue targeting, reducing off-target effects (useful in cancer therapy).	Requires precise engineering and may face challenges in consistent targeting efficiency.	[62]
Reduced toxicity	Lowers exposure to normal tissues, reducing side effects and improving patient tolerance.	Some formulations may trigger immune responses or rapid clearance from circulation.	[59, 63]
Controlled release	Enables sustained drug release, reducing dosing frequency and enhancing patient compliance.	Risk of premature drug leakage, affecting efficacy.	[59, 64]

11. LIPOSOME-LOADED DRUG DELIVERY SYSTEMS IN CANCER

Liposomes can address many of the common challenges faced by cancer immunotherapies. The unique characteristics of nanotechnology, particularly in the fields of drug delivery, diagnosis, and imaging, have spurred increased research into its application in cancer therapy. Numerous nanocarriers have been explored in preclinical and clinical research, and those that exhibit improved biocompatibility, targeted delivery, and controlled drug release show great promise for therapeutic applications. Liposomes, polymer micelles, inorganic nanoparticles, drug conjugates, and virus-like nanoparticles are some of the many types of nanocarriers [28].

Most liposomal drug formulations are suitable for intramuscular (i.m.) and intravenous (IV) administration. These formulations have been employed in various clinical trials for purposes, such as immunization and cancer targeting. In clinical settings, liposomal formulations like Doxil® and Depocyt® are already being used to treat cancer [26]. The components of Doxil® liposomes include N-(carbonyl-methoxy polyethylene glycol 2000)-high phase-transition-temperature (Tm), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), and hydrogenated soy phosphatidylcholine (HSPC), a phospholipid in a molar ratio of 56:38:5 [27].

This formulation allows for increased drug retention in tissues while decreasing drug efflux in circulation, which maintains appropriate levels of drug transport [28]. Depocyt® (Enzon Corporation, Piscataway, NJ, USA) contains cytarabine (Ara-C) as its active ingredient. Depocyt® is designed to manage neoplastic meningitis (NM) by controlling the release of Ara-C. The formulation encapsulates the aqueous medication solution in multivesicular nanoparticles known as DepoFoam, which is a slow-release formulation. DepoFoam™ technology, which encapsulates hydrophilic compounds like Ara-C, uses small spherical particles (3–30 μm) consisting of 4% biodegradable lipid and 96% water foam [29]. The remote drug loading technique for Doxil® permits the accumulation of roughly 15,000 DOX molecules per vesicle inside the liposome's hydrophilic core. To ensure stability, most of the drug (>90%) is stored as a crystalline-like deposit, which remains unaffected by osmotic forces [30]. Clinical trials have demonstrated that Doxil® minimizes cardiotoxicity, a common adverse effect of free DOX treatment, because the encapsulation prevents doxorubicin from accessing the myocardium and heart muscle cells [31, 32].

12. LIPOSOMES FOR DISORDERS CAUSED BY FUNGUS INFECTIONS

Liposomes are tiny, multi- or unilamellar vesicles composed entirely of cholesterol and phospholipids, providing a wide range of uses for drug delivery. Depending on their size, composition, lamellarity, and surface charge, nanosized liposomes function as efficient topical medication delivery systems to the skin. Dermal delivery involves targeting medications to a specific area beneath the skin, enhancing local pharmaceutical effects while minimizing systemic side effects [33]. The first study using liposomes for drug delivery to the skin was conducted by Mezei and Gulasekharam in 1980, with an emphasis on improving skin deposition and localizing pharmaceutical medicines. Encapsulating antifungal medicines in liposomes has been shown to improve epidermal penetration and localization, while also reducing percutaneous absorption. Furthermore, liposomes containing natural lipids and cholesterol are generally non-immunogenic [33].

Patients with leukemia, lymphoma, or immunodeficiency disorders like AIDS are at heightened risk of systemic fungal infections, often caused by *Candida* and *Aspergillus* species [32]. Amphotericin B (AmB) is the drug of choice for treating these infections, but its use is limited due to acute and chronic toxicity. Researchers have discovered that encapsulating AmB in liposomes significantly reduces its toxicity while preserving its therapeutic efficacy [34].

One such formulation, Abelcet®, is an intravenous (IV) suspension of AmB in a lipid complex. Developed by Sigma-Tau Pharmaceuticals in 1995, Abelcet® is designed to treat invasive fungal infections that are resistant to standard AmB desoxycholate treatment, or when traditional AmB therapy is contraindicated due to renal impairment or intoler-

able toxicity. Abelcet® features a 1:1 drug-to-lipid molar ratio, with two phospholipids, DMPG and DMPC, or dimyristoyl phosphatidylcholine and glycerol, respectively, forming the lipid complex in a molar ratio of 7:3. Even though Abelcet® contains a high concentration of AmB, the medicine is quickly absorbed into the body during infusion. Studies on pharmacokinetics reveal that the reticuloendothelial system (RES) deposits the drug, and lipase activity likely facilitates the release of AmB at local infection sites [35, 36].

Ambisome® is another liposomal formulation of AmB, approved in 1997 by Astellas Pharma USA for intravenous infusion. Ambisome® is used to treat severe, potentially fatal fungal infections, such as leishmaniasis, aspergillosis, blastomycosis, coccidioidomycosis, and a particular type of HIV-related meningitis [37]. Compared to Abelcet®, Ambisome® exhibits high clearance and a substantial distribution volume, suggesting substantial tissue absorption. Its slow clearance from the body leads to a prolonged terminal elimination half-life. Despite this, after repeated doses, AmB concentrations in the blood remain low [37]. In animal models with systemic fungal infections, Ambisome® demonstrated improved safety, better therapeutic tolerance, and a higher therapeutic index compared to traditional AmB [37].

In Ambisome®, electrostatic complexes are formed between the negatively charged distearoylphosphatidylglycerol (DSPG) and the positively charged mycosamine moiety of amphotericin B (AmB). Additionally, AmB is securely integrated into the liposomal membrane through hydrophobic interactions with cholesterol in the lipid membrane. In a 2:1:0.8:0.4 molar ratio, hydrogenated soy phosphatidylcholine, cholesterol, DSPG, and AmB make up the lipid bilayer of Ambisome® [38]. Ambisome® has a longer plasma retention time than conventional AmB, which results in increased plasma levels, although its pharmacokinetic profile differs from lipid complex formulations. Despite its slow clearance, Ambisome® predominantly accumulates in tissues associated with the mononuclear phagocyte system (MPS), such as the liver and spleen [39].

13. LIPOSOMES FOR GENE THERAPY

Gene therapy has shown great promise as a treatment for both acquired and genetic disorders. It offers potential solutions for various diseases, including hereditary conditions, certain types of cancer, and some viral infections. However, the widespread success of gene therapy is hindered by the absence of reliable delivery systems, both viral and nonviral [40]. Liposomes present a significant opportunity in this area due to their versatile ability to integrate multiple functions within a single system, offering desirable properties and functionality for gene delivery [41]. In gene therapy, the delivery of nucleic acids to cells is crucial for both *in vitro* and *in vivo* research. While *in vitro* methods can rely on various physical and chemical processes to facilitate nucleic acid transport, *in vivo* delivery is more challenging and requires additional optimization. DNA-carrier systems, which include a range of colloidal particles, have been explored for this purpose. Notably, cationic liposomes have demonstrated the ability to form complexes with negatively charged DNA. These complexes are capable of transfecting cells *in vitro*, enabling the expression of proteins encoded by the DNA plasmid. *In vivo*, the application of cationic lipid-based DNA complexes has been particularly promising. When administered locally, such as through intratracheal instillation in lung epithelial cells, or systemically, targeting lung endothelial cells, these complexes can effectively transfect specific cells and deliver gene therapy [42].

Gene therapy has also shown promise for treating specific breast cancer subtypes that are associated with various genetic abnormalities [34]. However, despite the approval of some gene therapy treatments, viral vector-based therapies have not proven to be effective for cancer treatment. Factors, such as age at menstruation onset, reproductive history, hormone use, lifestyle factors (like alcohol consumption, smoking, and diet), and genetic predisposition (*e.g*., family history and gene mutations), contribute to the complexity of breast cancer, which makes successful viral vector-based therapies challenging. For gene transfer applications, including gene therapy and vaccination, liposomes are often preferred over viruses. Unlike viruses, liposomes are non-immunogenic and simpler to assemble, making them an attractive alternative for gene therapy, particularly in the context of cancer treatment [41].

Liposomes have been used in clinical trials as a delivery vehicle for gene therapy in breast cancer. The clinical trial data are presented in Table **4**.

14. LIPOSOMAL VACCINES APPLICATIONS

Liposomes have been studied for decades as both vaccine adjuvants and antigen delivery systems, with their potential in vaccine delivery first being explored in 1974. Their widespread appeal as a vaccine delivery technology is primarily due to their flexibility and versatility. More recently, liposomes have been investigated for their ability to combat SARS-CoV-2. Specifically, surface-linked liposomal peptides have been explored for use as a potential vaccination against SARS-CoV-2, utilizing cytotoxic T cells (CTLs). Ohno S. *et al*. discovered four SARS-CoV-derived HLA-A*0201-restricted CTL epitopes. The liposomes used for delivery were composed of dioleoyl phosphatidyl ethanolamine, dioleoyl phosphatidyl choline, dioleoyl phosphatidyl glycerol acid, and cholesterol in a molar ratio of 3:4:2:7 [43].

15. CHALLENGES WITH DRUG DELIVERY ASSISTED BY LIPOSOMES

Liposomes provide tremendous opportunities in the field of drug delivery. However, liposome-based drug formulations have yet to make a significant impact on the market. The development and production of liposomes have been limited by challenges, such as stability, batch-to-batch consistency, sterilization processes, minimal drug entrapment, difficulty in controlling particle size, excessive batch sizes, and the rapid circulation half-life of the vesicles [44].

Table 4. Liposomes used in clinical trials as a vehicle for gene therapy for breast cancer.

Title of Clinical Trial	Status	Phase of Study	Strategy	Target Patients	Investigators	References
NGN201 (Ad5CMV-p53) Administered Locally in Patients with Locally Advanced Breast Cancer in Combination with Doxorubicin and Docetaxel: A Phase II Study	Completed, 2004	Phase II	Treatment of patients with stage III or stage IV breast cancer using a combination of the Ad5CMV-p53 gene therapy and liposomal chemotherapy agents, including docetaxel and doxorubicin hydrochloride.	Patients with stages III and IV breast cancer who are at least 18 years old (male or female).	Jill Van Warthood (Introgen Therapeutics), United States, Texas	[41]
An Investigation on the Effects of SGT-53 in Metastatic Triple-Negative Inflammatory Breast Cancer Using Carboplatin and Pembrolizumab	Started on 30th October, 2021	Phase I	Pembrolizumab, carboplatin, and transferrin receptor-targeted liposomal p53 cDNA may aid in the management of triple-negative inflammatory breast cancer patients.	Female patients with inflammatory breast cancer who are at least 18 years old.	Massimo Cristofanilli, FACP (Northwestern University), United States, Illinois	[41]

15.1. Stability

One of the key challenges to the general acceptance of liposomes is their physical and chemical stability. Due to their physical and chemical fragility, the final liposome formulations may not last very long. Instability can arise through ester bond hydrolysis or the oxidation of unsaturated acyl chains in lipids. Physical instability, on the other hand, may arise from drug leakage, as well as vesicle aggregation or fusion, which leads to the formation of larger particles. Both drug leakage and changes in liposome size can significantly impact the *in vivo* performance of the drug formulation, potentially affecting its therapeutic index [44].

15.2. Sterilization

Since the majority of liposome formulations are meant for parenteral administration, where sterility is necessary, sterilization is an important stage in the liposome production process. However, because of their sensitivity and vulnerability to physicochemical changes, liposome sterilization is still difficult. The most common method for sterilizing liposome formulations after manufacturing is filtration through sterile 0.22 μm membrane filters [44]. Despite the fact that filtration is widely used, it is time-consuming and ineffective at eliminating viruses [45]. Additionally, filtration must be performed under aseptic conditions, and the process can be expensive, requiring high-pressure equipment that may exceed 25 kg/cm² [46]. While γ-irradiation is effective, liposomes cannot be sterilized using it, although it can sterilize a variety of drugs and surgical tools. Liposomes' unsaturated phospholipids are vulnerable to instability and peroxidation when exposed to radiation due to free radical generation. Furthermore, this method requires high-pressure conditions (25 kg/cm² and above) and is generally only feasible on a large scale. These factors can lead to liposome degradation under irradiation [47].

15.3. The Production Costs of Liposomes

The cost of liposomes can be substantial, and functionalizing them on an industrial scale presents significant challenges. Additional concerns include the complexities of commercial-scale manufacturing, biological barriers to liposome delivery in humans, biosafety evaluations by health regulatory bodies, and the long-term storage requirements of liposomes, all of which contribute to high expenses. Currently, there are no standardized protocols for assessing the safety of liposomes or other nanodrugs. Innovative liposomal formulations may not be widely adopted due to the high costs of scaling up production. Additionally, the evolution and clinical translation of liposomes are constrained by the lack of established regulations and guidelines governing quality assurance, safety and efficacy assessments, and manufacturing processes [48].

15.4. Changes in Physicochemical Characteristics

At greater scales, aggregation, surface area, size, and shape might affect interactions with cells and biomolecules as well as biodistribution, making safety evaluations much more challenging. Furthermore, changes to the production process, reagents, synthetic method, or administration route may impact the toxicity profile and necessitate a reassessment of the drug's safety [48].

15.5. Large-Scale Production Challenges

Liposomes must be generated on a large scale with regular consistency in order to be used in human patients. Due to

Table 5. List of liposome and lipid-based drug products approved for human use and currently available in the market.

Brand	Drug	Route of Injection	Lipid Composition	Company	Indication	References
Doxil	Doxorubicin	Intravenous. (i.v.)	HSPC:Cholesterol: DSPEPEG2000 (11.2: 7.8:1)	Johnson & Johnson	Chemotherapeutic	[49]
Abelcet	Amphotericin B	-	DMPC: DMPG (2.3:1)	Leadiant Biosciences, Inc	Antifungal	[50]
Inflexal V	Influenza virus antigen, strains A and B	Intramuscular (i.m.)	Strains A and B of the influenza virus, which are 70% lecithin, 20% cephalin, and 10% phospholipids (DOPC: DOPE, 3:1)	Johnson & Johnson	Vaccine	[51]
DepoDur™	Morphine sulfate	Epidural	DepoFoam™	SkyPharma	Analgesic	https://doi.org/10.2165/00003088-200645120-00002 [52]
Visudyne®	Verteporfin	i.v.	Verteporphin:DMPC&EPG (1:8)	Novartis AG	Photodynamic therapy	https://doi.org/10.2147/ijn.s26766 [7]
Ambisome	Amphotericin B	i.v.	HSPC: DSPG: Cholesterol: Amphotericin B (5:2:2.5:1)	Fujisawa Healthcare, Inc. and Gilead Sciences, Inc.	Antifungal	https://pubmed.ncbi.nlm.nih.gov/14982807/ [53]
Arikayce	Amikacin	Oral inhalation	Amphotericin B with DPPC: 0.6–0.79: 1 weight ratio)	Insmed, Inc. of Bridgewater, NJ	Antibacterial	[54] [55]
Mepact	Mifamurtide	i.v.	DOPS: POPC (1:2.3)	Takeda Pharmaceutical Limited	Immunomodulator/ Antitumor	[56]
DepoDur	Morphine sulfate	Epidural	Tricaprylin and Triolein (507:11:76:6:1), DOPC, DPPG, and cholesterol	Pacira Pharmaceuticals, Inc.	Narcotic Analgesic	https://doi.org/10.1213/01.ane.0000265533.13477.26 [57]
Onivyde	Irinotecan	i.v.	DSPC: Cholesterol:MPEG2000-DSPE (3:2:0.015)	Merrimack Pharmaceuticals	Anti-cancer	[58]

issues with repeatability or scaling up production, many liposomal compositions never make it to the market. The difficulties of producing liposomes on a wide scale are increased by their complexity. For preclinical and clinical studies, liposomes have usually been made in small batches, which makes formulation management and modification simpler [48].

Several liposome- and lipid-based products approved for human use are currently available on the market, and the corresponding drug brands are listed in Table **5**.

16. FUTURE DIRECTION OF LIPOSOME PREPARATION

The future of liposome preparation will focus on automation, improved stability, cost-efficiency, enhanced drug loading, and regulatory advancements. While traditional methods like thin-film hydration and sonication face scalability challenges, automated microfluidic systems and 3D-printed liposomes will enable precise control and personalized formulations. Stability remains a crucial factor, but freeze-dried (lyophilized) liposomes and stabilizers, such as PEGylation and cholesterol derivatives, will extend shelf life and minimize drug leakage. The use of plant-based and biodegradable lipids, along with nanotechnology-driven self-assembling lipids, will help lower costs and improve accessibility. Remote drug loading techniques and AI-driven formulation design will enhance drug encapsulation efficiency, optimizing drug release and therapeutic efficacy. Smart liposomes, including pH-sensitive and temperature-responsive variants, will enable controlled, targeted drug delivery, while hybrid liposomes integrating biopolymers and lipids will enhance biocompatibility. Although regulatory challenges have slowed clinical adoption, the implementation of standardized quality control measures and streamlined regulations will ensure consistent and safe production. Increased clinical trials and investment will accelerate the integration of liposomal formulations into mainstream medicine, broadening their use in cancer therapy, gene delivery, and vaccine development. These innovations will transform liposome technology, mak-

ing it more scalable, cost-efficient, and clinically applicable, ultimately advancing drug delivery systems and improving patient outcomes.

18. DISCUSSION

Liposomes provide a versatile and biocompatible drug delivery platform that may encapsulate a wide range of therapeutic substances while allowing for controlled, targeted release. Formulation advances, such as PEGylation and ligand-based surface modifications, have improved stability, increased circulation time, and improved site-specific delivery, especially in oncology and gene therapy. Despite these gains, significant challenges remain. Manufacturing complexity remains a significant barrier, with concerns of scalability, reproducibility, and quality control. Innovative techniques, such as microfluidics, show promise, but additional refinement is required for industrial scale production. Regulatory constraints also impede clinical translation. Liposomal products require precise physicochemical characterization and consistent performance, however regulatory criteria differ between jurisdictions, complicating approval processes.

Overall, while liposomes have demonstrated strong therapeutic potential, overcoming these formulations, regulatory, and safety barriers is essential. Continued innovation in scalable production methods, harmonized regulatory pathways, and immunological profiling will be critical for their successful clinical integration.

CONCLUSION

With their effectiveness in targeted therapy, cancer treatment, vaccine development, and gene therapy, liposomes have become a viable drug delivery technology. Their stability, drug loading capacity, and therapeutic potential have all increased as a result of advancements in formulation procedures. Their clinical use is nevertheless hampered by a number of important issues, including production complexity, regulatory compliance, and immune response limitations. For them to be widely adopted, these obstacles must be removed via scalable production techniques, regulatory alignment, and optimized formulation strategies. In order to improve the clinical viability of liposome-based treatments, this review discusses recent advances in detail and highlights key areas for future research.

AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: study conception and design: MR; data collection: MR; data analysis or interpretation: SP, VS, and MR; methodology: MR and VS; investigation: MS, NK, SF, CK, and SJ; draft manuscript: MR. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

CL	=	Cardiolipin
CMC	=	Critical Micelle Concentration
DLS	=	Dynamic Light Scattering
DOPC	=	1,2-dioleoyl-sn-glycero-3-phosphocholine
DOPE	=	1,2-dioleoyl-sn-glycero-3-phosphoethanolamine
DOPG	=	Dioleoyl Phosphatidylglycerol
DPPC	=	Dipalmitoyl Phosphatidylcholine
GUVs	=	Giant Unilamellar Vesicles
LUVET	=	Large Unilamellar Vesicles produced by Extrusion Techniques
LUVs	=	Large Unilamellar Vesicles
MLVs	=	Multilamellar Vesicles
MULs	=	Medium Unilamellar Vesicles
NM	=	Neoplastic Meningitis
PA	=	Phosphatidic Acid
PALS	=	Phase Analysis Light Scattering
PC	=	Phosphatidylcholine
PE	=	Phosphatidylethanolamine
PG	=	Phosphatidylglycerol
PI	=	Phosphatidylinositol
PS	=	Phosphatidylserine
SUVs	=	Small Unilamellar Vesicles
ULV	=	Unilamellar Vesicles

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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