REVIEW PAPER

Harnessing liposome technology for precision medicine: design, delivery, and clinical impact

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ABSTRACT

Background: Liposomes are microspheres formed by phospholipids and have received attention as sophisticated drug carriers because they can retain both water-soluble and fat-soluble drugs. Their applications have made them very versatile and have resulted in their great exploration in many aspects of therapy.

Objective(s):The present review sets out to provide an exhaustive overview of the nature of liposomes, their preparation tactics, measures of their efficiency, and the trending applications of liposomes in contemporary medicine, with a focus on optimization measures and the future of this technology.

Materials and Methods: The mini-review of the literature was done with an emphasis on the various methods of liposome preparation, which included the Bangham method, solvent injection methods, as well as the removal methods of detergents. Efficiency was analyzed based on optimization and evaluation parameters, including vesicle size, shape, zeta potential, and in vitro release profile of the drug to evaluate their contribution to formulation efficiency.

Results: Recent innovations have resulted in the creation of new generation liposomal systems, such as active targeting liposomes, stimuli-reactive liposomes, and surface-modified liposomes. These advances bring substantive benefits to drug delivery effectiveness and outcomes in the therapeutic arena and decreased systemic toxicities. Liposomal formulations find increasing use in cancer treatment, in the treatment of infectious diseases, and in gene delivery. A number of novel systems are in the clinical trial stage, indicating their translational potential.

Conclusion: Liposomes are a very flexible and evolving drug delivery mechanism. The development of improved surface modification, targeting approaches, and stimuli-responsive systems still augers well with the therapeutic potential. Continuing advances in the development and clinical assessment of new liposomal preparations demonstrate the future of these agent-carrying constructs to transform the field of disease management in the domains of cancer, infectious disease, and gene therapy.

Keywords: *Antineoplastic agents, Drug delivery systems, Gene therapy, Liposomes, Nanomedicine*

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INTRODUCTION

In Liposomes, "lipo" means “fat," & "soma" means "body." Constructively, they have two-layered vesicles. The first publication on the subject was released in 1964, stating that phospholipids and other amphiphilic lipid molecules in solution self-assemble to create liposomes, which are colloidal spherical structures. The liposomal membrane contains one or multiple phospholipid bilayers (lamellas) surrounding an internal aqueous area where polar head groups face internal and external water phases (Fig.1). The layered structure of liposomes allows them to carry and move molecules that do not easily dissolve because they are uniformly structured [1]. The lipid bilayer contains hydrophobic molecules, the interior aqueous core contains hydrophilic molecules, and the water/lipid bilayer interface contains amphiphilic molecules. Because they are water-attracted, the heads group together to create a surface facing the water wherever there is any. The tails group creates an out-of-water surface because they are water-repellent. There is one layer of heads attracted to water in the environment and pointing towards the exterior of the cage [2].

Another layer of heads is turned inward, attracted by the water within the cell. Because of their diverse shapes, liposomes have been studied more than any other carrier system.

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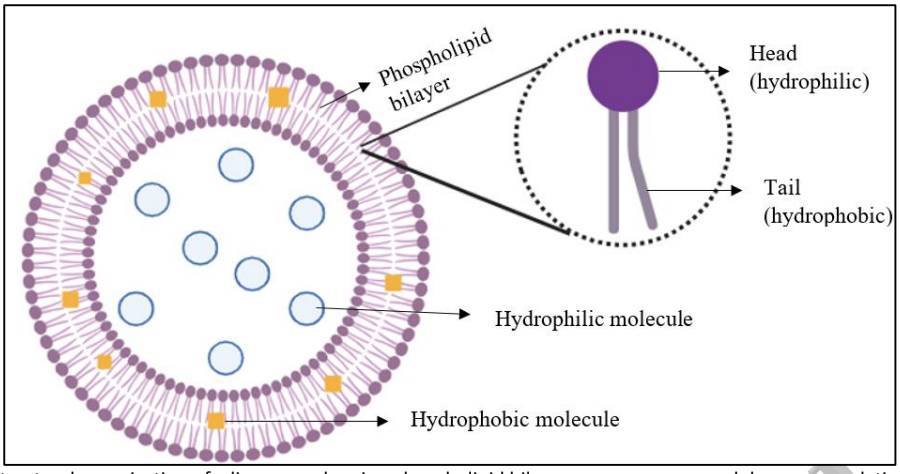

Fig.1. Structural organization of a liposome showing phospholipid bilayer, aqueous core, and drug encapsulation sites.

By adding phospholipids to a water solution, phospholipid bilayer membranes can form sphere-shaped structures known as liposomes that include internal hydrophilic compartments. Small, spherical vesicles called Phospholipids, cholesterol, and non-toxic detergents are all able to encapsulate liposomes [3]. Even for membrane proteins, the investigations led to the consideration of liposomes as delivery systems, which are distinguished by the presence of a diverse range of chemicals in the core region. Both hydrophilic and hydrophobic chemicals can be adversely delivered and encapsulated by these devices. Because they improve the bio-distribution and stabilize medicinal substances, Liposomes as drug delivery technology enhance therapy for many medical conditions and eliminate obstacles for cellular and tissue uptake of chemicals to target tissues *in vivo* [4]. This is because the liposome shields the drug from the physiological processes that include enzymatic degradation, chemical and immunological inactivation, and rapid clearance from plasma, enhancing and prolonging its efficacy. As the therapeutic agent is entrapped inside the liposome, its contact with normal tissue is reduced compared to the free drug, thus reducing the risk of adverse side effects [5].

Optimization in the process of liposome formulation and testing

Optimization plays an important role in liposome studies in order to achieve high entrapment efficiency, as well as stability, controlled release, size reduction of the vesicles, and biocompatibility. Traditional methods of trial-and-error learning are ineffective; thus, Design of Experiments (DoE) and Response Surface Methodology (RSM) are used [6-10].

Factorials design (full & fractional factorial)

Research design in which many factors (formulation variables) are studied at once at several levels. In a 2^2 factorial design, there exist 2 factors which are run in two levels (low and high) hence resulting in 4 experimental runs.

Digits meaning: A 3^2 design is a two-factor statistical experimental design with three levels (low, medium, and high) of each factor, making a total of 3^2 =9 experimental runs. The former digit (3), which is found in this notation, signifies the number of levels within each of the factors, and the superscript (2) shows the level of factors under investigation. It is the common design used in a full factorial experiment to systematically research the effects of several factors and their interaction with a response variable.

Use in liposomes: Liposomes are used in an initial screening of variables such as the phospholipid-to-cholesterol ratio, hydration time, or sonication power. It aids in the discovery of important variables that may affect the size of the vesicles, zeta potential, as well as entrapment efficiency.

Central Composite Design (CCD)

Response surface design (RSD) Expanding on a factorial design are the center points and axial (star) points added to assess reproducibility of a factor and explore the curvature of surfaces. A common CCD consists of three components: factorial points (a 2-level factorial design), center points, and axial points that enable one to explore extreme values. In a 2-factor CCD, each factor will have five levels ($-\alpha$, -1, 0, +1, $+\alpha$). In this case, the design can support quadratic modeling. This allows CCD to be a well-used tool in applications involving optimizing liposomes, where we can model linear and quadratic effects of parameters on dependent

responses such as bilayer rigidity, the rate of drug release, and stability.

Box Behnken design

A Box Behnken design (BBD) is a dataset of an RSM that is less costly as compared to a CCD, based on its ability to require fewer experimental runs and does not employ extreme axial points. It is designed by the midpoints of the design space edges and the center points, and factors were studied in three levels (−1, 0, and +1). The main benefit of BBD is that it does not cause severe conditions in an experiment, but this aspect may be highly beneficial in cases where such conditions may result in unstable products such as liposomes. With liposomes, a BBD would be suitable in investigating the collective effects of the concentration of the lipid, the drug-to-lipid ratio, and the rate of stirring on key properties such as the size of the vesicles and polydispersity index.

Taguchi design

A robust design. A Taguchi robust design is a method of design that uses the concept of orthogonal arrays to effectively screen the interactions of many factors on a process or product in a much smaller number of runs than would be needed to study a wide range of interactions in a full factorial design. The use of such an approach in the context of liposomes would be very beneficial to a preliminary screening of many of the process parameters, such as the pH, hydration medium, and sonication time, and can point out which variables are most influential by requiring the least effort.

Plackett–Burman design

Plackett-Burman design (PBD) is a screening design to be used to separate the most important variables out of many possible variables. The reason is that it considers in evaluations of the main effects of each factor and not the interactions, hence it is efficient. Within the context of liposomes, a PBD can be useful to reduce the key formulation variables, such as type of lipid or surfactant concentration, to consider first with the aid of a more complex optimization approach, e.g., a CCD or a BBD.

Table 1 provides a comprehensive summary of various optimization techniques utilized in the formulation and evaluation of liposomes, highlighting their principles, methodologies, and significance in enhancing liposomal stability, drug entrapment efficiency, and therapeutic efficacy.

Table 1. Summarize different optimization techniques used for the formulation and evaluation of liposomes.

Drug	Design	Independent variables	Dependent variables	Ref.
Oridonin	BBD (Box Behnken design)	Ethanol (X_1), extraction time (X_2), and solid/lipid (X_3).	Entrapment efficiency (Y_1), particle size (Y_2), polydispersity index (Y_3), and zeta potential (Y_4)	[11]
5- fluorouracil	3^2 factorial design (FFD)	Drug: lipid (X_1), Ascorbyl-6-palmitate (X_2).	Particle size (Y_1), entrapment efficiency (Y_2), and zeta potential (Y_3).	[12]
Rifampicin	3^2 FFD	Soya lecithin /cholesterol (X_1).	Particle size (Y_1) and entrapment efficiency (Y_2).	[13]
Lycopene	3^3 FFD	Soy lecithin (X_1), and cholesterol/ β-CD (X_2).	Particle size (Y_1), entrapment efficiency (Y_2), drug release (Y_3), and zeta potential (Y_4).	[14]
Amisulpride	2^3 FFD	Cyclodextrins (CDs) (X_1), drug/CDs (X_2), and liposomes (X_3).	Particle size (Y_1), entrapment efficiency (Y_2), polydispersity index (Y_3), and zeta potential (Y_4).	[15]
Glipizide	3^2 FFD	Paraffin wax (X_1) and the stearic acid in the wax(X_2).	Particle size (Y_1), entrapment efficiency (Y_2), and drug release (Y_3)	[16]
Bufalin	3^3 FFD	Bufalin: lipid (X_1) and cholesterol: EPC (X_2).	Particle size (Y_1), zeta potential (Y_2), and entrapment efficiency (Y_3).	[17]
Pingyangmycin	BBD	PYM (X_1), CS (X_2), and GP (X_3).	Drug release (Y_1) and release rate constant (Y_3).	[18]
Acyclovir	3^3 FFD	The vol. of the organic phase (X_1), the vol. of the aqueous phase (X_2), and Drug/Phosphatidylcholine /Cholesterol (X_3).	Entrapment efficiency (Y_1).	[19]
Paeonol	BBD	Soybean phosphatidylcholine: Cholesterol (X_1), paeonol (X_2), pH of PBS(X_3).	Entrapment efficiency (Y_1).	[20]
Ophiopogon polysaccharide	BBD	Soybean phosphatide/ OP (X_1), soybean phospholipid/ cholesterol (X_2), and chloroform to a phosphate-buffered saline (X_3).	Particle size (Y_1) and zeta potential (Y_2).	[21]

Drug	Design	Independent variables	Dependent variables	Ref.
Epimedium	3^4 FFD	Drug to lipid (X_1), soybean phospholipid/ cholesterol (X_2).	Entrapment efficiency (Y_1) and Drug-loading (Y_2).	[17]
Travoprost	$3^1 \times 2^1$ FFD	Type of permeation enhancer (PE) (X_1), PE (X_2), and lecithin/ cholesterol (X_3).	Entrapment efficiency (Y_1), particle size (Y_2), polydispersity index (Y_3), and zeta potential (Y_4).	[22]
Temozolomide	3^2 FFD	Lipid/ organic phase (X_1).	Particle size, entrapment efficiency (Y_2), and drug loading (Y_3).	[23]
Ginsenoside	BBD	Lipid to drug (X_1), ePC to cholesterol (X_2), Lipid (X_3).	Entrapment efficiency (Y_1).	[24]
Primaquine	BBD	Phospholipid type (X_1), cholesterol (X_2), charge (X_3), and citrate (X_4).	Entrapment efficiency (Y_1) and particle size (Y_2).	[25]
Methazolamide	3^2 FFD	Cholesterol (X_1) and drug (X_2).	Entrapment efficiency (Y_1) and particle size (Y_2).	[26]
Liposome polycation-DNA complexes (LPD)	CCD (central composite design)	Protamine/DNA (X_1), Chems/ DNA (X_2), and Chems/ Dioleoylphosphatidylethanolamine (X_3).	Particle size (Y_1) and entrapment efficiency (Y_2).	[27]
Piroxicam	CCD	HLB (X_1), Total Lipid amount (X_2), and Surfactant: cholesterol (X_3).	Entrapment efficiency (Y_1) and drug release (Y_2).	[28]
Besifloxacin hydrochloride	3^2 FFD	Soya lecithin: cholesterol(X_1), and lipid: drug (X_2).	Entrapment efficiency (Y_1), drug loading (Y_2), and Particle size (Y_3).	[29]
Bovine Lactoferrin	2^4 FFD	Percentage of cholesterol in lipid content (X_1), drug (X_2), surfactant (X_3), and sonication time (X_4).	Particle size (Y_1) and Entrapment efficiency (Y_2).	[30]
Ganoderma lucidum polysaccharide	BBD	Soybean phosphatide/ cholesterol (X_1), soybean Phosphatide/ tween 80 (X_2), and ultrasonic time (X_3).	Entrapment efficiency (Y_1).	[31]
Doxorubicin and curcumin	BBD	Phospholipid (X_1), curcumin (X_2), Doxorubicin (X_3), Working temperature (X_4), Buffer pH (X_5), and Phospholipid: cholesterol molar ratio (X_6).	Entrapment efficiency of curcumin (Y_1), Entrapment efficiency of doxorubicin (Y_2), and zeta potential (Y_3).	[32]
Quercetin	D-optimal experimental design	dipalmitoyl phosphatidylcholine (DPPC) (X_1), DPPC: Cholesterol (X_2), and quercetin (X_3).	Drug loading (Y_1), polydispersity index (Y_2), and entrapment efficiency (Y_3).	[33]

Benefits of liposomes

Liposome delivery presents several benefits to the drug delivery process, and it increases the efficacy, shelf life, and safety of several drugs. The liposomes can overcome these effects and reduce the accompanying side effects of toxicity to healthy tissue by encapsulating the chemotherapeutic compounds so that, at optimum doses, the accompanying toxicity to healthy tissue is reduced, as well as protecting against enzymatic axerophagy. This flexible system can encapsulate a broad chemical composition, including water-soluble, amphiphilic, lipid-soluble drugs, and even big fragments of DNA. Also, the construction of liposomes can reduce exposure of the cytotoxic drugs to sensitive tissue, and the shuttles can be used to carry either positively or negatively charged drugs. They may be specifically designed to target selected cells or tissue, and are themselves non-toxic, biocompatible, and biodegradable [34-36].

Drawbacks of liposomes

Although liposomes are very efficient in drug delivery, they still possess a number of disadvantages. Their reduced stability is a major concern, which will cause leakage of the encapsulated drug or molecule and subsequent fusion of the vesicles. Also, although the internal compartment (inside the liposome) is aqueous, the lipid bilayer itself is not water-soluble. The liposome is also composed of phospholipids that may experience degradation in the form of dephosphorylation, hydrolysis, and oxidation, further disrupting the stability. These aspects make liposomal formulations have a short half-life in the body. Lastly, they are extremely costly to produce, and as such, they may restrict their usage on a larger scale [37, 38].

Types of liposomes

Grounded on structure

Table 2 provides a detailed overview of the different types of vesicles, categorizing them based on their size ranges and lamellarity while explaining their structural characteristics, formation methods, and applications in drug delivery and biomedical research.

Table 2. Types of vesicles with their size ranges and lamellarity [39].

Vesicle type	Size	Lamella
Unilamellar vesicle (ULV)	All sizes range	1
Small Unilamellar Vesicle (SUV)	0.02-0.1µm	1
Medium Unilamellar Vesicle (MUV)	>0.1µm	1
Large Unilamellar Vesicle (LUV)	>0.1µm	1
Giant Unilamellar Vesicle (GUV)	>1µm	1
Oligolamellar vesicle (OLV)	0.1-1µm	5
Multilamellar vesicle (MLV)	>0.5µm	5-25
Multi-vesicular vesicle (MVV)	>1µm	1

Grounded on the methods of preparation

Liposomes are spherical vesicles with phospholipid bilayers, prepared using the Bangham method, sonication, extrusion, reverse-phase evaporation, and solvent injection (Fig.2). Each technique affects size, entrapment efficiency, and stability, optimizing them for drug delivery applications [40, 41].

Different liposomes with their composition

The composition of the liposomes is described [42, 43] (Fig.3).

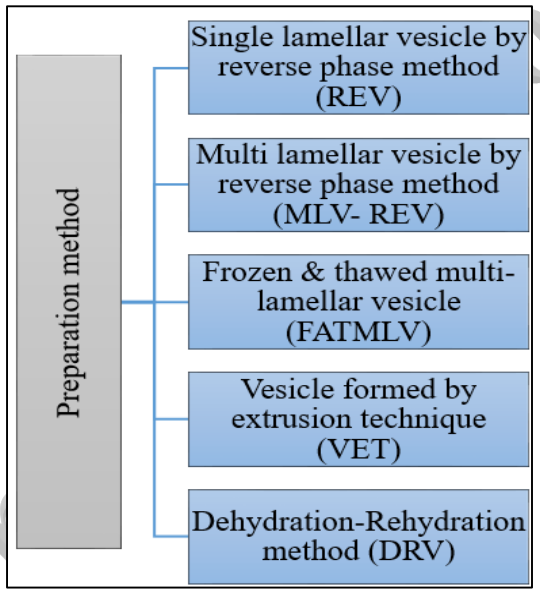

Fig.2. Types of Liposomes Based on Preparation Methods like Multilamellar Vesicles, Small Unilamellar Vesicles, Large Unilamellar Vesicles, and Giant Unilamellar Vesicles.

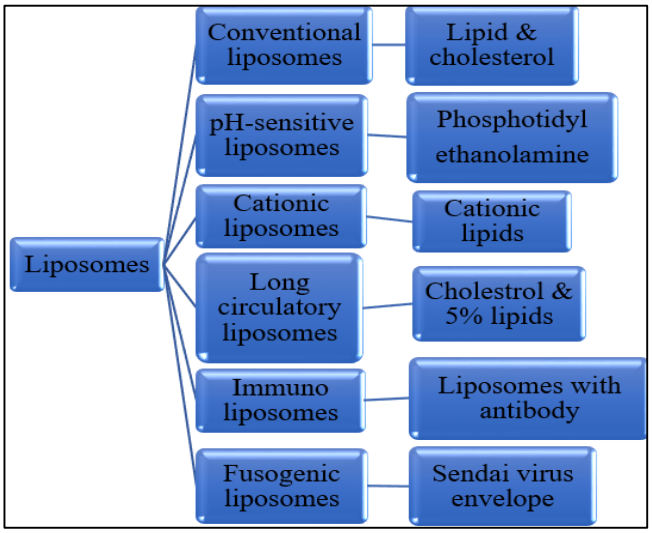

Fig.3. Different Types of Liposomes with Their Composition, like Conventional Liposomes, Stealth Liposomes, Cationic Liposomes, Immunoliposomes, and Stimuli-Sensitive Liposomes.

MATERIALS AND METHODS

The successful incorporation of medication, along with the achievement of monodisperse patches with controlled size distribution and desired lamellar arrangement, forms a basis for stable colloids over time and is the primary claim of a system for liposome nano formulation conformation. The traditional methods involve dissolving liposomes in an unexpected organic detergent and combining them with a waterless phase. The stability of the produced nano-formulation may be impacted, or the chemical composition of the integrated active composites may be disturbed by the presence of an organic detergent. The following key steps are involved in the traditional liposome medication styles [44]:

- Lipids dissolve in an organic detergent.
- The organic detergent's accompanying lipidic outcome is dried down.
- Using a waterless medium to hydrate the lipid, then stirring or moving it
- Diminished size (and/or altered lamellarity)
- Processing after formation (sterilization, sanctification)
- The final nano formulation product's characteristics

Bangham method

A round-bottom flask dissolves a hydrophobic drug and lipids in an organic solvent, forming a thin film that evaporates under reduced pressure. A heated aqueous buffer hydrates the film, incorporating hydrophilic drugs into the liposome's interior. Slower hydration improves entrapment efficiency. Liposome scaling, lamellarity, and size distribution are controlled via sonication or membrane extrusion, with extrusion preferred for stability and efficiency. While sonication aids SUV liposome production, it can degrade lipids or drugs and may cause metal contamination (Fig.4) [45].

Ethanol injection method

Ethanol-solubilized phospholipids are rapidly injected into a heated buffer, causing self-assembly as ethanol dilutes. This leads to lipid precipitation, forming bilayer fragments that merge into unilamellar vesicles upon solvent depletion. Ethanol content is crucial, ≤7.5% ensuring homogenous SUVs, while rapid addition to excess buffer produces MLVs. Residual ethanol is removed via dialysis and filtration, with spontaneous SUV and LUV formation. Ethanol evaporates at room temperature (Fig.5) [46].

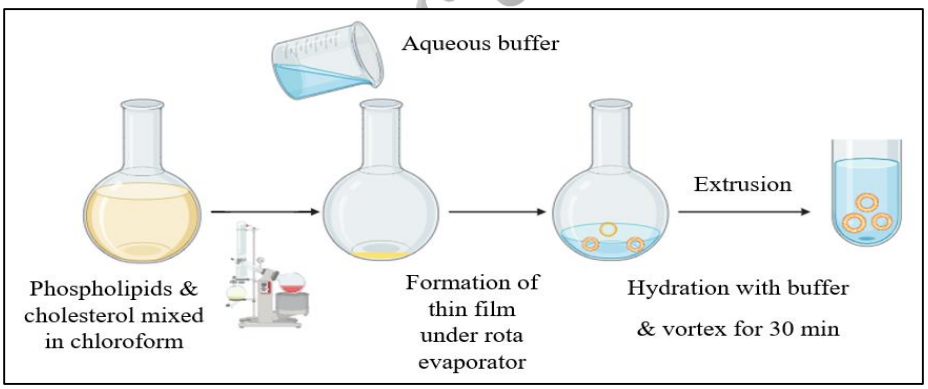

Fig.4. Liposome Preparation Using the Bangham Method by hydrating the thin lipid film formed by evaporating the solvent.

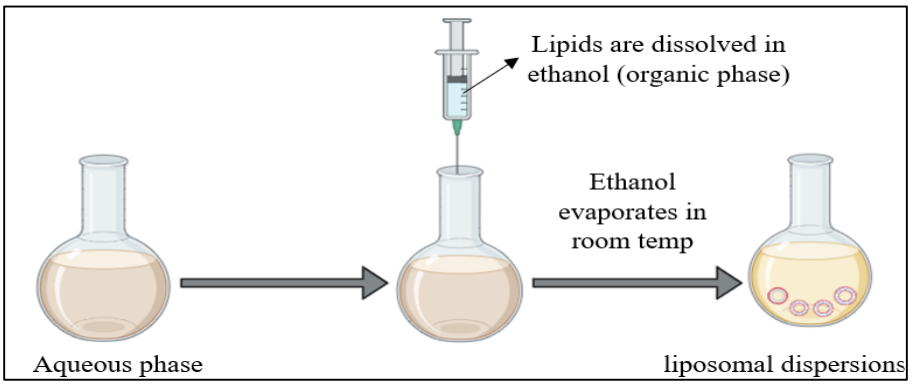

Fig.5. Preparation of Liposomes by Ethanol Injection Method, which involves a lipid solution in ethanol that is rapidly injected into an aqueous phase under stirring, leading to spontaneous formation of small unilamellar vesicles.

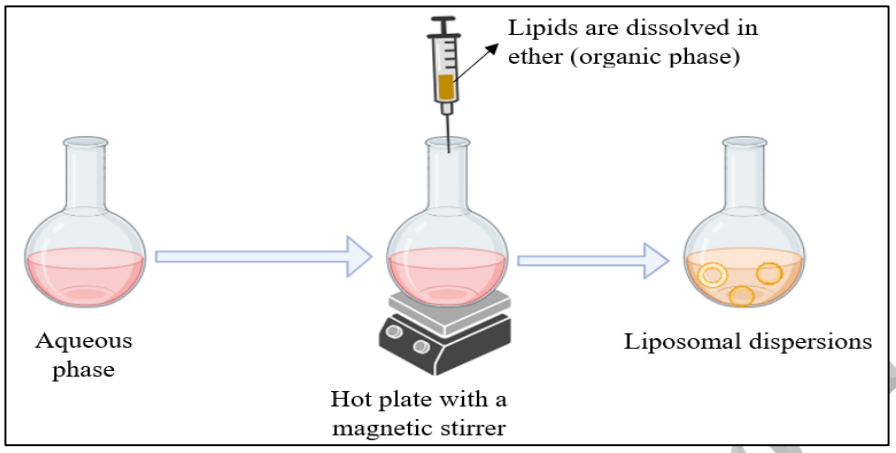

Fig.6. Preparation of Liposomes by Ether Injection Method that involves a lipid solution in volatile ether is slowly injected into an aqueous phase at 55–65 °C, causing ether evaporation and the gradual formation of liposomes.

Ether injection method

In the ether injection method, lipids in ether are slowly added to an aqueous phase containing encapsulated ingredients and then heated (55–65°C) to evaporate the solvent. Slow evaporation under reduced pressure favors LUV formation, while SUVs arise via ether vaporization. This method removes solvents more effectively than ethanol injection, improving liposome concentration and entrapment efficiency. However, it results in polydisperse liposomes and exposes active agents to solvents and heat, potentially affecting stability and safety (Fig.6) [47].

Solvent- evaporation method

Solvent- phase evaporation relies on inverted micelle formation, enabling high aqueous space-to-lipid ratios and efficient encapsulation. Hydrophilic substances are enclosed in a buffered aqueous phase, while amphiphilic molecules in an organic phase are sonicated to form micelles. Slow solvent removal increases viscosity until micelles disrupt, forming bilayers around the remaining micelles. This method produces liposomes with more aqueous content than MLVs. Sonication of a phospholipid suspension creates an oil-water emulsion, which, under reduced pressure, forms a gel that transitions into liposomes upon further solvent removal. It achieves high entrapment efficiency in low-ionic-strength media but risks denaturing proteins or shearing DNA due to sonication and solvent exposure [48].

Detergent removal method

In the detergent removal method, lipids are hydrated and solubilized in a detergent solution, forming mixed micelles. As the detergent is gradually removed, lipid-enriched micelles transition into unilamellar vesicles. The most convenient removal method is dilution (10–100 fold) with a buffer, causing micelles to grow, become polydisperse, and eventually form vesicles beyond the micellar phase boundary. In lecithin-bile salt systems, dilution reduces bile salt concentration, decreasing monolayer curvature and promoting liposome formation. Liposomes emerge when detergent levels fall below the critical micelle concentration (CMC), but low liposome concentration and inefficient entrapment of lipid-soluble contents limit the process [49].

The dehydration-rehydration method

This involves rehydrating said SUVs in the void volume with the material-containing aqueous fluid of interest to entrap and immediately dry. Consequently, the solid lipids are finely fractured and dispersed. There are various methods, but freeze-drying is the most common one. The vesicles are then rehydrated. The oligolamellar vesicles (OLV) were obtained with this method (Fig.7) [50].

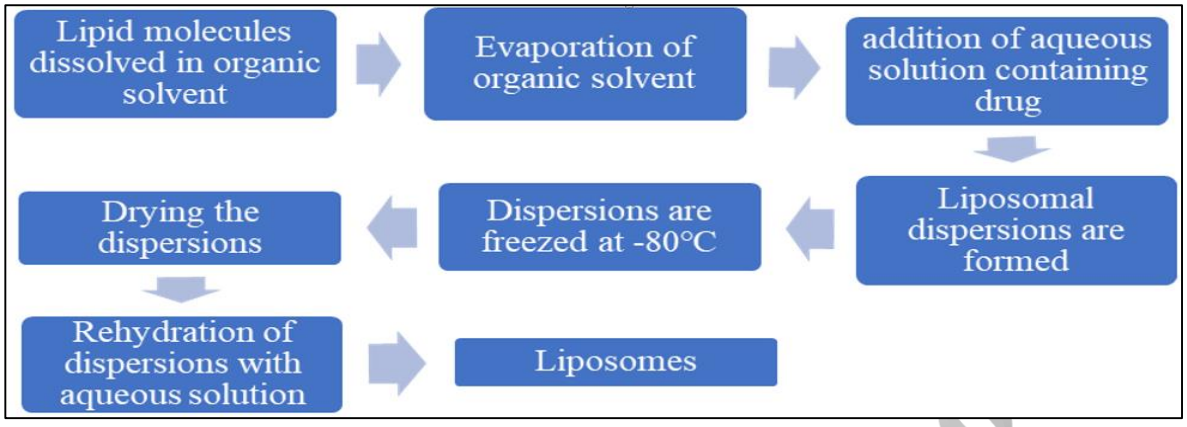

Fig.7. Scheme of Preparing Liposomes by the Dehydration–Rehydration Method

Proliposome method

The pro-liposomal approach is a simple method for liposome production with high entrapment efficiency but low repeatability. For small-scale preparation, lipids are dissolved in ethanol and water, stirred at 60°C for 10 minutes, cooled, then mixed with water or buffer to form MLVs, which hydrate for an hour. For large-scale production, a lipid-ethanol-buffer mixture (1:1:2) is stirred at 60°C, cooled, and hydrated dropwise. MLVs are further hydrated via sonication. Liposomes are stored under nitrogen gas at -80°C or 4°C. Lipophilic molecules are added in ethanol, while hydrophilic molecules are incorporated in the aqueous phase(Fig.8) [51].

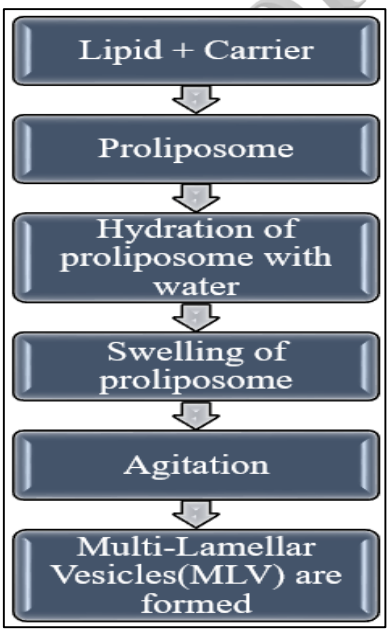

Fig.8. Detailed process of liposome preparation through the proliposome method for enhanced stability and encapsulation efficiency

Heating method

It is a non-organic solvent-based process. Lipid Hydration Direct hydration of lipids with an aqueous solution containing a 3-5 per cent hydration agent such as glycerin or propylene glycol, which is then heated to the melting point of the phospholipids used for at least an hour, is the method adopted for liposome production. If cholesterol is added to the composition, the suspension can be heated to 100 °C, and the hydrating agents will act as stabilizers and isochronizing additives to stop the lumping and settling of nanoparticles. Furthermore, the heat treatment is efficient in forming powder-inhalable liposomes due to the cryoprotective action of the hydrating agents (Fig.9) [52].

Microfluidic channel method

The microfluidic channel method is a recent technique of liposome preparation that has been introduced. Microfluidics provides a means of using liquids in thin channels. In this method, as per the technique, lipids are dissolved in a suitable organic solvent, and the solution is injected into the microchannels standing or against the flow of the aqueous medium. The aqueous and organic solutions are mixed axially continuously in the method to create liposomes. Surfactants are used to stabilize liposomes, preventing their separation and coagulation. The production of consistent liposomes with desired characteristics, such as average size, polydispersity, morphology, and lamellarity, is achieved through microfluidic channel techniques that regulate the blending of aqueous and organic phases [53].

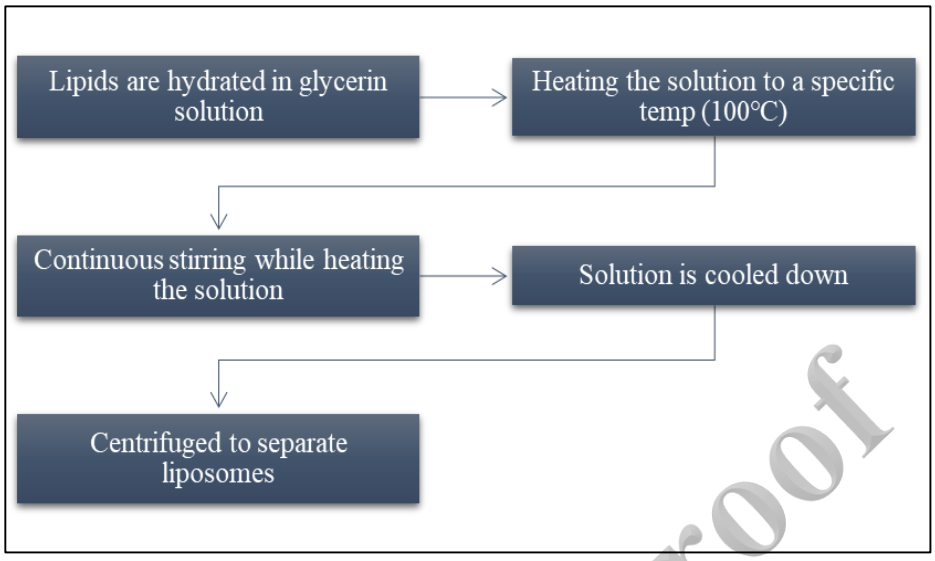

Fig.9. Stepwise process of liposome preparation using the heating method for improved vesicle formation and stability

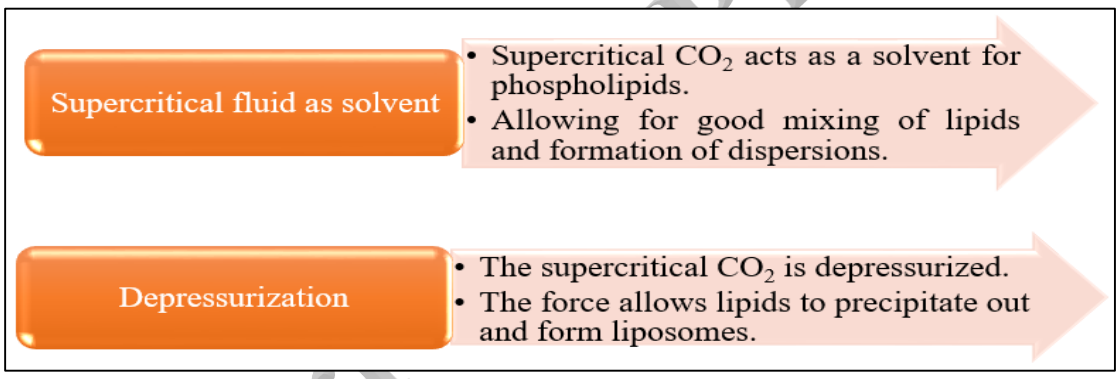

Fig.10. Stepwise process of liposome preparation using the supercritical fluid method for enhanced particle size control and stability

Supercritical fluidic method

Instead of employing organic detergents, this technique uses lipids that are solubilized using CO_2, which is a supercritical fluid. A high-pressure liquid pump continuously introduces the water-free phase into a cell where the supercritical lipid product can be delivered so that the phase transformation of solubilized phospholipids is facilitated. After all the CO_2 is removed, the liposomes would be created by an instantaneous drop in pressure. This system achieved a 5-fold improvement in encapsulation edge. This system uses inexpensive, environmentally safe carbon dioxide; however, it has unique designs, excessive costs, and low yields (Fig.10) [54].

pH jumping method

The pH jumping method is an additional solvent-free technique for liposome production. This approach breaks down MLVs into SUVs by subjecting the phosphatidic acid and phosphatidylcholine aqueous solution to a pH increase of four times in a short amount of time. SUV production rates differ from LUV rates because of the relative concentrations of phosphatidic acid and phosphatidylcholine (Fig.11) [55].

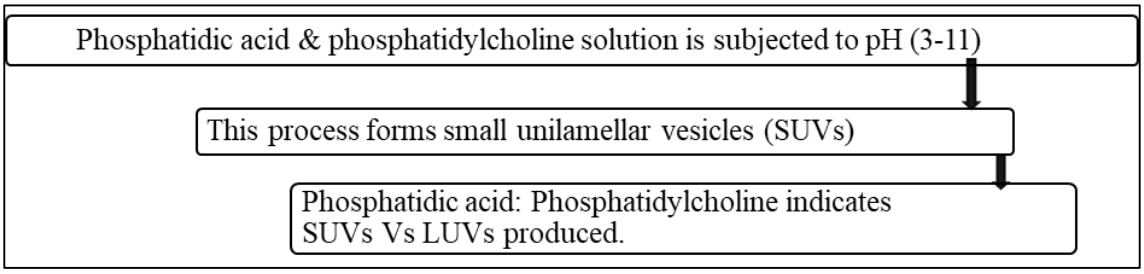

Fig.11. Stepwise process of liposome preparation using the pH jumping method for controlled vesicle formation and stability

Evaluations of liposomes

Vesicle shape

Liposome morphology is best examined using microscopy. Electron microscopy, particularly TEM, allows direct visualization but requires sample alteration, making it time-consuming and unsuitable for routine use. TEM may also distort liposome shape. Cryo-TEM, using liquid nitrogen flash-freezing, preserves liposomes' original form but is more effective for smaller nanoparticles. AFM enables non-destructive, high-resolution 3D imaging without sample manipulation, making it a faster and more effective alternative to electron microscopy [56].

Vesicle size & polydispersity index

Key characteristics for liposome characterization are size and polydispersity index (PDI). Size affects circulation time; smaller liposomes (50–200 nm) last longer, while larger ones are cleared faster. PDI (<0.3) indicates uniformity, with higher values suggesting heterogeneous populations. Dynamic light scattering (DLS) is the primary technique for measuring both, utilizing Brownian motion and light scattering to determine size, but struggles with particle separation and contamination sensitivity. Nanoparticle tracking analysis (NTA) tracks individual particle movement to calculate size and concentration, complementing DLS for verification [57].

Zeta potential (ZP)

Zeta potential measures the net charge on liposomes, influencing electrostatic interactions and stability. It depends on lipid composition, head groups, and environmental factors like ionic strength. Low zeta potential increases aggregation risk, while highly charged liposomes repel each other, enhancing stability. Measurement involves laser illumination of the sample, analyzing scattered light changes under an electric field to determine electrophoretic mobility. Using the Henry equation, the zeta potential is calculated. Capillary electrophoresis and laser Doppler electrophoresis (LDE) are the primary methods for measurement [58].

Lamellarity

Another property that may influence future liposomal applications is lamellarity because it affects the EE and drug release characterization. The most widely employed method, cryo-TEM, gives useful information on the lamellarity of liposomes, e.g., their bilayer thickness and bilayer-to-bilayer distance. Other techniques for determining lamellarity are based on differences in the visible or fluorescence response of lipid markers following the addition of specific reagents. The ^{31}P NMR method has been employed to assess the value of lamellarity for liposomes, specifically the outer-to-inner phospholipid amount ratio. Paramagnetic ions in sample preparation for the NMR samples quench the ^{31}P NMR signal of the lipids. As the contact of ions with the bilayer changes the NMR spectrum, the lamellarity of liposomes can be approximated by comparing the two spectra before and after the addition of paramagnetic ions. The other methods that can be employed to approximate the lamellarity of liposomes are SAXS and trapped volume determination [59].

Phase behavior

Phase behavior is essential in drug delivery as increased lipid membrane fluidity enhances hydrophilic drug permeability. It also affects liposome fusion, aggregation, stability, and protein interactions. The transition temperature is primarily studied using Differential Scanning Calorimetry (DSC), which measures heat flow differences between a sample and reference under controlled conditions. Other methods include Thermogravimetric Analysis (TGA), fluorescence probe polarization, electron paramagnetic resonance, nuclear magnetic resonance, FTIR spectroscopy, X-ray diffraction, and molecular dynamics simulations [60].

Entrapment efficiency

Entrapment efficiency (EE) is influenced by bilayer rigidity, liposome composition, and production methods. It measures the percentage of the drug encapsulated within liposomes compared to the total drug used. EE quantification involves separating free (unencapsulated) drugs via centrifugation or ultracentrifugation, followed by drug measurement in liposomes. Indirect methods subtract unencapsulated drugs from the total, while direct methods dissolve lipids in organic solvents for measurement. Drug quantification techniques include protein/enzyme assays, UV-Vis and fluorescence spectroscopy, and advanced methods like GC, UPLC, HPLC, ^{1}H NMR, and ESR [61].

In vitro drug release

Dialysis conditions help determine the in vitro drug release profile. The dialysis membrane must allow free drug permeation without adsorption interference. A liposomal sample is sealed in a

dialysis bag and submerged in a pH 7.4 buffered saline medium at 37°C with agitation to simulate physiological conditions. At set intervals, aliquots are removed, analyzed, and replaced with fresh medium to maintain volume. The release profile is plotted as a cumulative release over time, aiding in designing liposomes for controlled drug delivery [62].

Table 3 provides a comprehensive overview of various analytical techniques employed for the assessment of liposomes, detailing their principles, methodologies, and significance in evaluating critical parameters such as size, surface charge, entrapment efficiency, stability, and drug release profiles.

Table 3. Different analytic techniques are used for the assessment of liposomes.

Evaluation test	Analytic techniques
Particle Shape	TEM (Transmission electron microscopy), AFM, and cryo-TEM.
Particle Size	Atomic force microscopy (AFM), and transmission electron microscopy (TEM).
Zeta potential	Laser Doppler electrophoresis (LDE) and Capillary electrophoresis.
Lamellarity	Cryo-TEM
Phase behavior	DSC (differential scanning calorimetry) and thermogravimetric study (TGA), fluorescence probe polarization, NMR, Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction (XRD).
Entrapment efficiency	HPLC, liquid chromatography with ultra (UPLC), liquid chromatography-mass spectrometry.
In vivo release	HPLC and UPLC.

FUTURE PERSPECTIVES

As an increasing number of drugs are safe to be transferred through the membranes of the liposomal outer layer, the liposomal vesicles have become one of the most popular methods to deliver drugs of various aspects, such as pain relievers and cancer treatments. There remain, however, several severe obstacles in their development and commercial production, such as the increased blood clearance of PEGylated liposomes, the inter-individual variability in the action of EPR (Enhanced Permeability and Retention) entities, and the reproducibility and control of excipients. These challenges notwithstanding, research is underway in developing many different new liposomal systems, including active targeting liposomes and stimuli-sensitive liposomes. One major multi-phase clinical failure was that of a thermosensitive liposome known as ThermoDox that failed during its Phase III trial in treating hepatocellular carcinoma in combination with radiofrequency ablation. Active targeting strategies are aimed at targeted targets not only in receptors on the cell surface of cancer cells or the tumor microenvironment but also in nonstandard stimuli, including pH, temperature, redox, enzymes, light, and ultrasound inducers of drug release. Coupling ligands such as proteins, peptides, carbohydrates, or monoclonal antibodies to the surface of liposomes and targeting tumor microenvironment components, such as integrins, matrix metalloproteinases, and vascular cell adhesion molecules, has also been studied. An important limitation is the nonhomogeneous population of tumor cells and expression of surface antigens, in which the applicability of ligand-based targeted therapies is restricted to a minority of tumors. The future Outlook Combinations of various ligands might be a promising solution to the detection and treatment of malignant lesions [63, 64].

CONCLUSION

Liposomes are the exclusive novel drug delivery systems, which have the potential to be used in controlled and targeted medicine delivery. Liposomes can be administered orally, parenterally, and topically, and in prolonged-release formulations. Liposomes solubilize lipophilic drug candidates; otherwise, they would be. Intravenous administration is challenging. Because phospholipids are lipophilic, liposomes can pass through the blood-brain barrier, so even hydrophilic medications can pass through with ease. Although there are several ways to make liposomes, the thin film approach, dehydration, rehydration, and many more are the most often employed techniques. The pharmaceutical industry has already seen the successful discovery, registration, and introduction of some commercial liposomes. Liposomal drug formulations have a broad range of therapeutic applications, from pain control to cancer treatment, and provide a way to get around the drawbacks of traditional medicines. Although there are still numerous barriers to achieving their full potential, increasing interest in creating medication formulations based on liposomes may encourage the creation of the next generation of liposomes as drug carriers, which would enhance patients' quality of life. This review has also briefly summarized the classification, preparations, evaluations, and different applications of liposomes. Upcoming developments in vesicular systems, particularly in cancer therapy, will be revolutionized by liposomal drug delivery systems.

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CONFLICT OF INTEREST

Author's declares no conflict of interest.

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