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Review Paper

Liposomes in Drug Delivery: An Updated Overview

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ABSTRACT

Liposomes are phospholipid-based vesicular systems capable of encapsulating both hydrophilic and lipophilic drugs. Since their discovery by Alec D. Bangham in 1961, they have become an important platform in drug delivery due to their biocompatibility, ability to modify pharmacokinetics, and potential to reduce toxicity. This review summarizes the structure, mechanism of action, classification, preparation methods, and evaluation parameters of liposomes. It also discusses their pharmaceutical application, marketed formulations, limitations, and recent developments of various types of novel liposomes. Liposomes continue to offer significant potential for improving therapeutic effectiveness across multiple routes of administration

INTRODUCTION

In 1961 British Hematologist Dr. Alec D. Bangham working at Babraham Institute in Cambridge, first identified liposomes. This discovery was made unexpectedly when he dispersed phosphatidylcholine molecules in water, leading to the formation of closed bilayer structure with an aqueous core surrounded by a lipid bilayer.[1] The term 'Liposomes' is derived from the Greek words 'lipos' meaning fat, and 'soma' meaning body. Liposome refers to a lipid body, which is a spherical microscopic vesicle made up of one or more concentric lipid bilayers, separated by water and aqueous buffer compartment, with diameters ranging from 25nm to 1000nm.[2] Liposomes are defined as "Simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule". Various amphipathic molecules are utilized to create liposomes. Drug molecules can be either encapsulated within the aqueous space or integrated into the lipid layer.[3]

Advantages[4,5]

1) Naturally free from ionic characteristics.
2) Completely biodegradable, non-toxic, biocompatible and non-immunogenic.
3) Capable of transporting both water- soluble and lipid- soluble drugs.

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4) Suitable for administering hydrophobic, amphipathic, and hydrophilic drugs.
5) Protects the encapsulated drug from external influences.
6) Reduces toxicity and enhances stability through encapsulation.
7) Increases effectiveness and therapeutic index.
8) Limits the exposure of sensitive tissues to toxic drugs.
9) Demonstrates site avoidance effects.
10) Improves protein stabilization.
11) Provides a sustained release over time.
12) Alter the pharmacokinetics and pharmacodynamics of drugs.

Disadvantages[4,6]

1) Short duration of effectiveness.
2) Poor solubility and reduced stability.
3) High production expenses.
4) Leakage and merging of encapsulated drugs or molecules.
5) Phospholipid may sometimes undergo reactions like oxidation and hydrolysis.
6) Rapid absorption by cells of the R.E.S.
7) Liposomal components can occasionally trigger allergic reactions.
8) Difficulty in targeting various tissues due to their large size.

STRUCTURE OF LIPOSOMES:

Liposomes consist of a lipid bilayer with a diameter ranging from 50 to 1000nm, functioning as a targeted delivery system that encapsulates active biological compounds.[7]

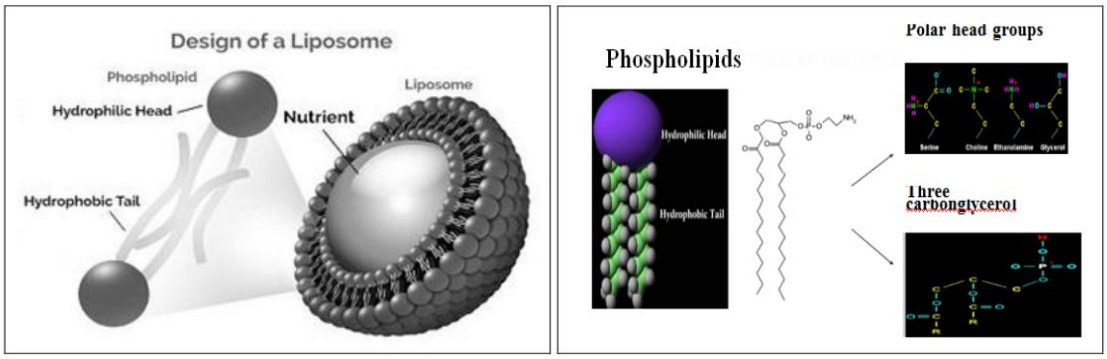


Fig 1: Structure of liposome [8]

1) Phospholipids

Phospholipids serves as the primary structural elements of liposomes. Phosphatidylcholine is one of most commonly used phospholipid in liposome formulation.[9]This amphipathic molecule comprises a hydrophilic polar head group called phosphocholine, a glycerol bridge and two hydrophobic acyl hydrocarbon chain. In its natural state, Phosphatidylcholine includes a glycerol moiety connected to two acyl chains, which may be either saturated or unsaturated.The arrangement of these hydrocarbon chains within the lipid molecules affects the stability of the liposome membrane.[10]The properties of the fatty acids in the lipid, such as number of double bonds, play a crucial role in determining the bilayer's elasticity and phase behavior. Phospholipids are prevalent in nature, and those containing choline are commonly used in the creation of liposomes.[11]

Examples of phospholipids: -

1. Phosphatidylcholine (PC)
2. Phosphatidylethanolamine (PE)
3. Phosphatidylserine (PS)
4. Phosphatidylglycerol (PG)

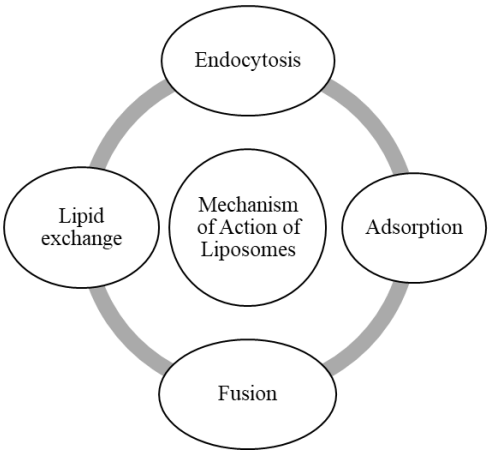
2) Cholesterol

Cholesterol serves as a crucial structural element in liposomes and is a widely utilized sterol. The inclusion of sterol influences the stability and rigidity of the structure. Cholesterol alone does not create a bilayer structure.[12] It integrates into

phospholipids at high concentration, reaching a molar ratio of up to 1:1 or 2:1 with phosphatidylcholine. The presence of cholesterol within the lipid bilayer boosts stability and forms a highly ordered and rigid membrane structure. [13] Cholesterol decreases the permeability of water-soluble molecules and enhances the fluidity and stability of biological membranes. It also prevents the interaction and destabilization of liposomes.[14]

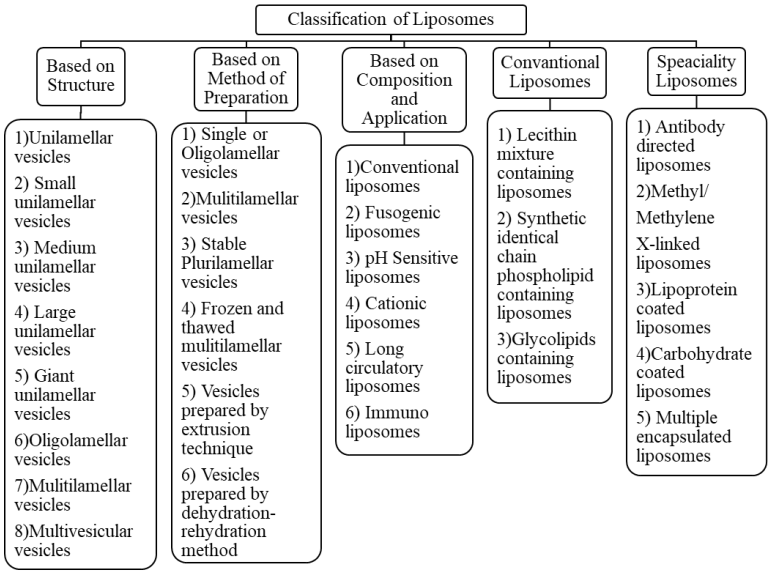
MECHANISM OF ACTION OF LIPOSOMES:

Liposome performs their action by four different mechanisms. They are as follows:



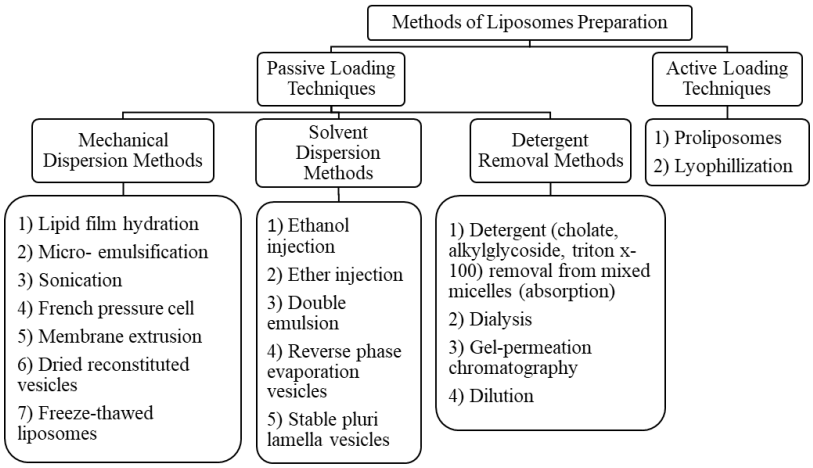
1) **Endocytosis**- This process is carried out by phagocytic cells within the reticuloendothelial system, such as neutrophils.[15]
2) **Adsorption**- This occurs on the cell surface through non-electrostatic forces or interactions with cell surface components.[16]
3) **Fusion**- This involves the integration of the liposomal bilayer into the plasma membrane, leading to the gradual release of liposomal contents into the cytoplasm.[17]
4) **Lipid exchange-** In this mechanism, liposomal lipids are transferred to the cellular membrane without the involvement of liposomal contents.[18]

CLASSIFICATION OF LIPOSOMES: [1,19,20]



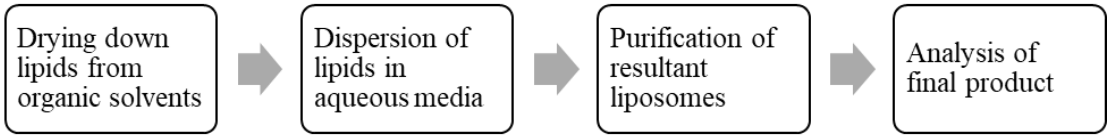
METHODS OF LIPOSOMES PREPARATION:[20]

There are different methods involved in the preparation of liposomes.



General Method of Preparation[21]

It involves four steps for the preparation of liposomes.



Liposomes can be prepared by passive and active loading techniques.

This technique involves loading of entrapped agents before or during the manufacturing process.[22]

A. Passive Loading Techniques

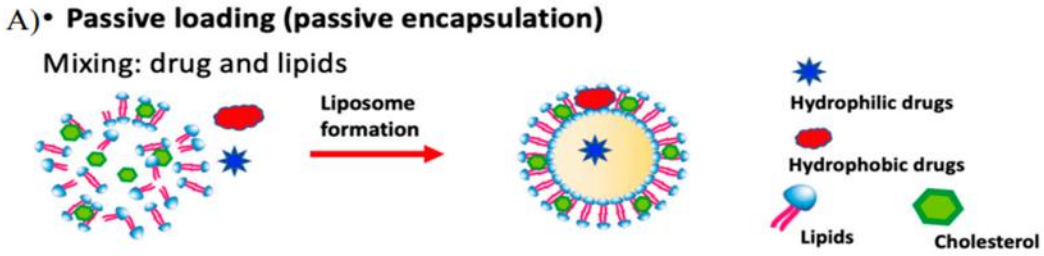


Fig 2:Passive loading technique[23]

a) Mechanical Dispersion Methods

1) Lipid film hydration method

This technique is used to produce MLVs. The process begins by dissolving phospholipids in an organic solvent, specifically Chloroform: Methanol, in a 2:1 v/v ratio, within a round bottomed flask. The flask is then attached to rotary evaporator, which rotates at 60 rpm, facilitating the evaporation of organic solvent. This results in the formation of a thin, uniform lipid film on the inner surface of the flask. To ensure all residual solvent is removed, nitrogen gas is applied. The lipid film is subsequently hydrated with an aqueous medium, creating a milky white suspension. This suspension is allowed to rest for

2 hours at room temperature or above the lipid's transition temperature, enabling the particles to swell completely and form MLVs.[24,25]

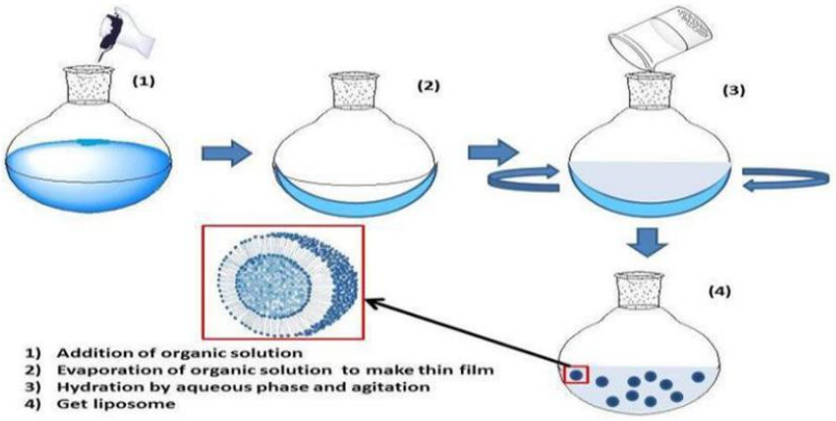


Fig 3: Lipid film hydration method[22]

2) Micro emulsification method

The micro emulsification technique produces small MLVs by utilizing a Micro fluidizer. In this process, lipids are either introduced as large MLVs or as a slurry of unhydrated lipids in an organic medium. The Micro fluidizer forces the fluid through a 5 µm orifice at a pressure of 10,000 psi. This high pressure causes the fluid to travel through microchannels, where two fluid streams collide at right angles with increased velocity. The resulting fluid is collected and cycled back through the pump and interaction chamber until spherical vesicles are formed. After just one pass, the vesicles size is reduced to a diameter of 0.1 to 0.2 µm.[25]

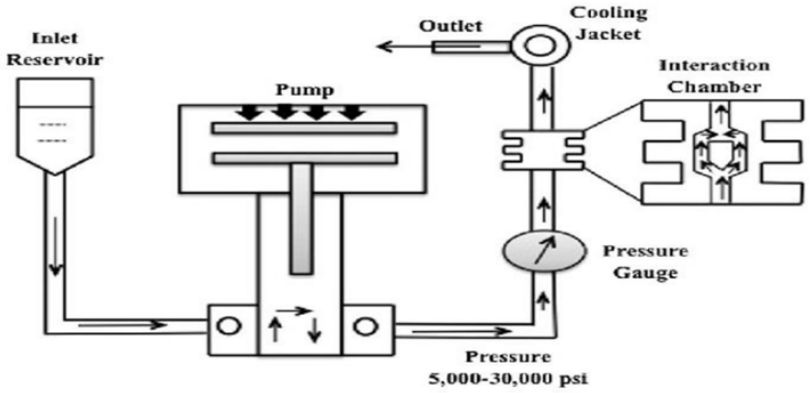


Fig 4: Micro emulsification method[26]

3) Ultrasonification method

This process used to transform MLVs into SUVs. It typically employs either bath or probe type sonicators within an inert atmosphere of nitrogen or argon.

Principle- The method uses pulsed, high frequency sound waves to agitate the MLV suspension.

a. Probe type sonicator

This technique involves a titanium probe that imparts high energy to the liposomal suspension.

Disadvantages- Thermo-sensitive substances such as proteins or DNA may become denatured or inactivated. Additionally, the liposomal suspension might degrade due to the release of titanium particles.

b. Bath type sonicator

To overcome drawbacks of probe type sonicators, bath type sonicators are mostly used. In this approach, a test tube with MLV dispersion is placed in a bath type soniactor. The MLV dispersion is sonicated for about 5-10 minutes at a temperature exceeding the lipid's transition temperature. The results in a slightly hazy transparent solution, which is then centrifuged to obtain SUV dispersion. During centrifugation, MLVs and titanium particles settle as sediment. The tube is then removed from the rotor, and the top clear liquid layer is carefully decanted using a Pasteur pipette, leaving behind the central layer with MLVs and pellet. The top layer contains the pure SUVs dispersion.[22]

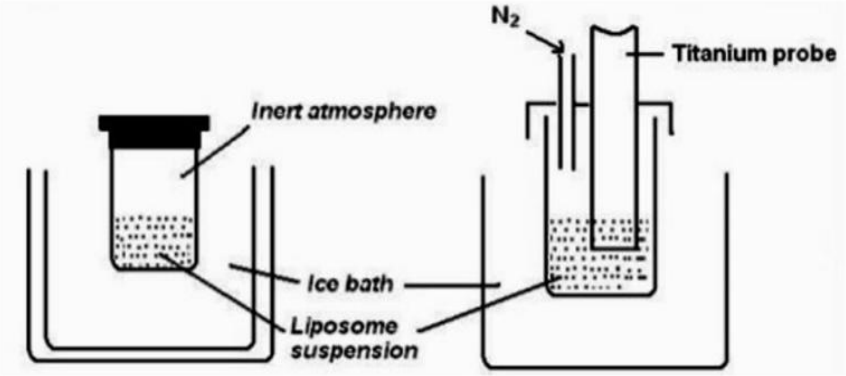


Fig 5: Bath and Probe type sonicators[26]

4) French pressure cell method

The French pressure cell, made up of stainless steel, is designed to withstand pressures between 20,000 and 40,000 psi. Its components include a pressure relief valve, a piston, a bottom seal, and a valve closure, with both the piston and bottom seal featuring rubber O-rings. This method is not only costly but also presents difficulties in cleansing the cell. The procedure starts with the introduction of a liposomal suspension into the pressure chamber, followed by the insertion of the piston. The cell is then rotated 180°. After filings, the bottom seal is pressed, and the chamber is sealed. The cell is then returned to an upright position and placed in a hydraulic press to generate pressure. Finally, the valve is opened slowly, allowing the liposomes to be released drop by drop. This process yields ULVs or OLVs with sizes ranging from 30 to 80 nm.[22]

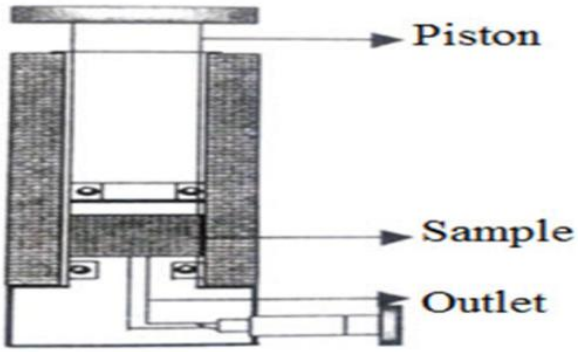


Fig 6: French pressure cell method[27]

5) High pressure extrusion method

This approach provides benefits such as high capacity and ease of use, along with quick processing. It is employed in the production of SUVs and LUVs. The process resembles the peeling of an onion. It utilizes polycarbonate filter membranes, through which MLV suspension is forced at a pressure of 250 psi. As this occurs, the layers are gradually removed, resulting in a single remaining layer. Consequently, liposomes of uniform size are produced. Liposomes created using this method are referred to as LUVETs.[22]

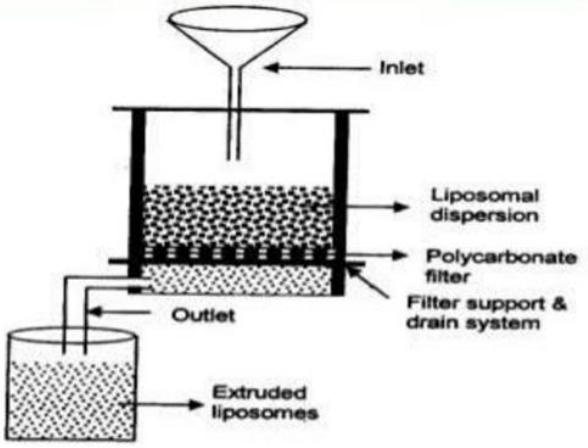


Fig 7: High pressure extrusion method[26]

6) Dried reconstituted vesicles method

The process begins by freeze-drying a dispersion of empty SUVs and then rehydrated it with an aqueous solution that contains the materials to be encapsulated. This results in the dispersion of solid lipids in finely subdivided form. Instead of drying lipids from an organic solution, freeze-drying is employed to freeze and lyophilize the performed SUVs dispersion. This method creates an organized membrane structure that, upon the addition of water, can rehydrate, fuse, and reseal to form vesicles with a high capacity for capturing materials. It is utilized for producing uni- or oligo-lamellar structures with diameters of 1.0 µm or smaller. The advantages include high entrapment of water-soluble content and the use of mild conditions for the preparation and loading of bioactive substances.[27]

7) Freeze thaw sonication method

This approach consists of a sequence of actions such as freezing, thawing, and sonication. Initially, a unilamellar dispersion (SUV) is frozen. Afterwards, the frozen dispersion is left to thaw at room temperature for approximately 15 minutes. Following this, sonication is performed. As a result, SUVs aggregate, leading to creation of LUVs.[22]

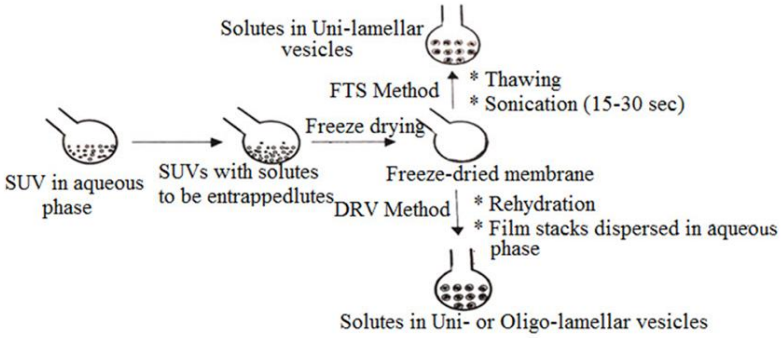


Fig 8: Dried reconstituted vesicles method and Freeze thaw sonication method[27]

b) Solvent Dispersion Methods

1) Ethanol injection method

Ethanol is employed to dissolve lipids, and the resulting solution is quickly injected through fine needle into an excess buffer solution, leading to spontaneous formation of SUVs. This method is limited to producing relatively dilute SUV suspensions. A significant issue is the removal of residual ethanol, which can be managed through ultrafiltration and vacuum distillation.[27]

In this method, lipids are dissolved in diethyl ether or ether methanol mixture and are slowly injected into an aqueous solution containing the materials can be encapsulated, at temperature ranging from 55 to 65°C. The subsequent vacuum removal of ether leads to the formation of liposomes. However, this method has limitations, such as producing liposomes of varying sizes (70-190 µm) and exposing compounds to organic solvents or elevated temperatures.[27]

2) Ether injection method

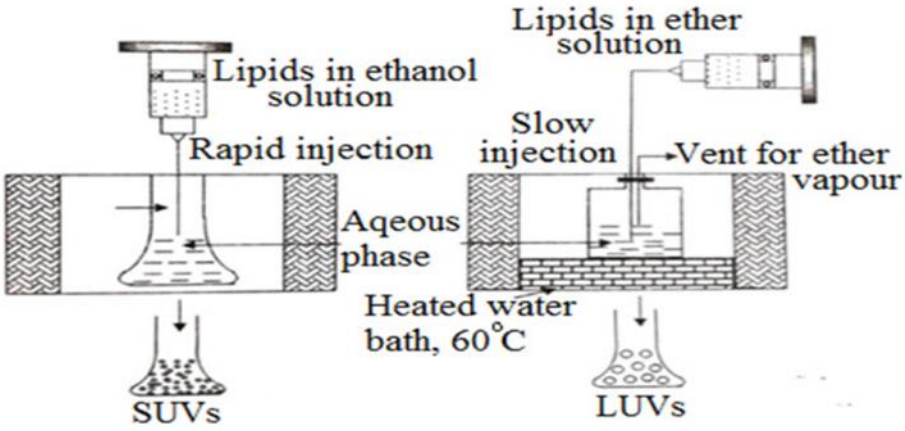


Fig 9: Ethanol injection method and Ether injection method [27]

3) Double emulsion method

In this approach, the initial step involves creating a primary emulsion by dissolving the drug in an aqueous phase (W1), which is then emulsified within a polymer's organic solvent to form a primary W1/O emulsion. This emulsion is subsequently combined with an emulsifier containing another aqueous solution (W2) to produce a W1/O/W2 double emulsion. Once the solvent is removed, microspheres remain in the aqueous continuous phase and are collected through filtration or centrifugation.[8]

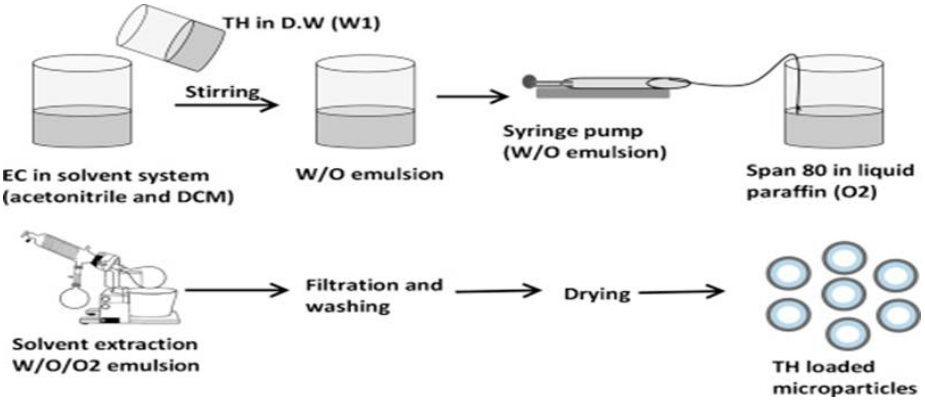


Fig 10: Double emulsion method[8]

4) Reverse phase evaporation vesicles method

This technique primarily involves the evaporation of solvent from an emulsion. Initially, lipids

dissolved in organic solvents are subjected to bath sonication, creating water-in-oil (W/O) emulsion. This emulsion is then concentrated into a semi-solid gel using rotary evaporator under reduced pressure. Subsequently, vigorous mechanical shaking with vortex mixture is employed to cause collapse of some water droplets, resulting in the formation of large unilamellar vesicles. The encapsulation efficiency can reach up to 50%.[27]

5) Stable pluri lamella vesicles method

In this method, W/O dispersion is created similarly to the reverse phase evaporation method but with an excess of lipids. During the drying process, continuous bath sonication is performed with a nitrogen stream. This allows for the redistribution and equilibration of the aqueous solvent and solute among the various bilayers within each plurilamellar vesicle. The entrapment efficiency is approximately 30%.[27]

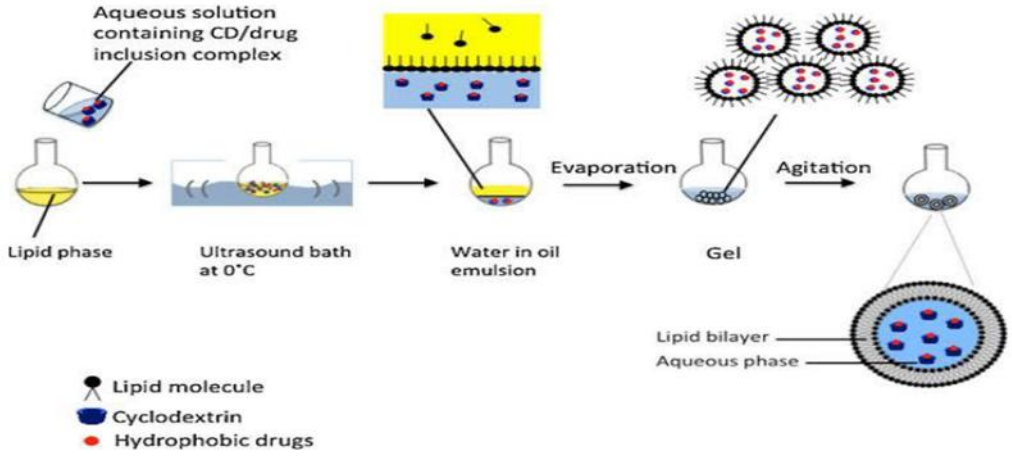


Fig 11: Reverse phase evaporation vesicles method and Stable pluri lamella vesicles method[26]

c) Detergent Removal Methods

1) Detergent (cholate, alkyl glycoside, Triton x-100) removal from mixed micelles (absorption)

Detergent absorption is achieved by agitating a mixed micelle solution with organic polystyrene bead absorbers like XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany) and Bio-beads SM2 (Bio-Rad Laboratories, Inc., Hercules, USA). The great benefit of using detergent absorbers is their ability to remove detergent with a very low critical micelle concentration (CMC), which are not completely eliminated otherwise.

2) Dialysis

Detergent at their critical micelle concentration (CMC) are employed to dissolve lipids. As the detergent is removed, the micelles become increasingly enriched with phospholipids and eventually merge to form large unilamellar vesicles (LUVs). The removal of detergents is achieved through dialysis. A commercial device named as LIPOPREP (Diachema AG, Switzerland), which is a type of dialysis system, is available for detergent removal. Dialysis can be conducted using dialysis bags immersed in large volumes of detergent-free buffers (equilibrium dialysis).

3) Gel-permeation chromatography

In this technique, detergents are removed by size-specific chromatography. Material such as Sephadex G-50, Sephadex G-100 (Sigma-Aldrich, MO, USA), Sepharose 2B-6B, and Sephacryl S200-S1000 (General Electric Company, Tehran, Iran) are suitable for gel filtration. Liposomes do not enter the pores of beads packed in the column but instead move through spaces between the

beads. At, slow flow rates, the separation of liposomes from detergent monomers is highly effective. The swollen polysaccharide beads absorb significant amounts of amphiphilic lipids, necessitating pretreatment. This pretreatment involves pre-saturating the gel filtration column with lipids using empty liposome suspensions.

4) Dilution

When an aqueous mixed micellar solution of detergent and phospholipids is diluted with buffer, the micellar size and polydispersity increase significantly. As the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from poly-dispersed micelles to vesicles occurs.(4)

B. Active Loading Techniques

This technique involves loading of certain type of compounds into the liposomes after the formation of intact.(22)

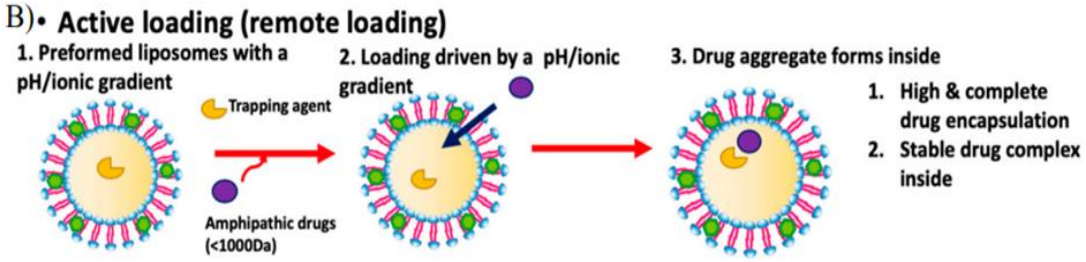


Fig 12: Active loading technique(23)

a) Proliposomes

This technique involves coating a soluble carrier with both lipid and drug to create a free-flowing granular material in pro-liposome which forms an isotonic liposomal suspension on hydration.(22)

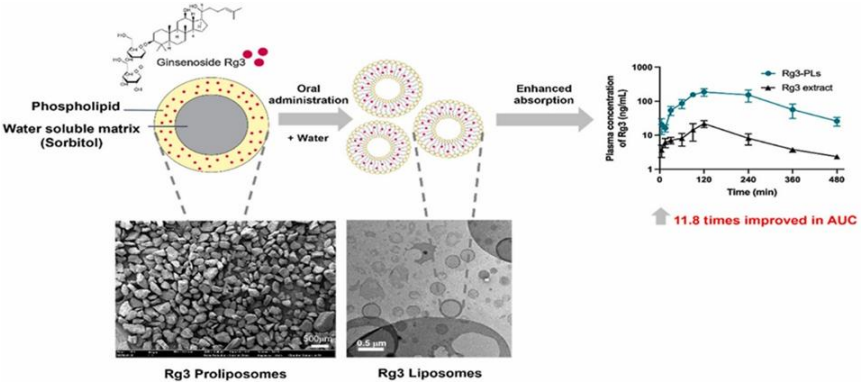


Fig 13: Proliposomes (28)

b) Lyophillization

This process, also known as freeze-drying, involves removing water from products while they are in a frozen state under reduced pressure. It is typically employed to dry products that are thermolabile.(22)

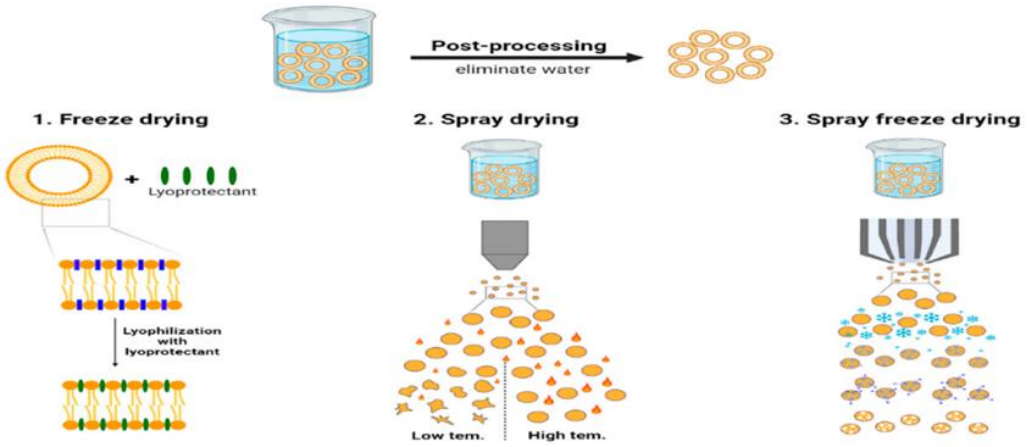


Fig 14: Lyophillization[29]

EVALUATION OF LIPOSOMES:[1,7,30]

Sr. No.	Parameters	Parameters	Techniques	Purpose
1.	Characterization of Liposome Structure	Morphology	Transmission Electron Microscopy (TEM)	High-resolution imaging of size, shape and lamellarity.
			Scanning Electron Microscopy (SEM)	Surface structure and morphology.
		Size and Size Distribution	Dynamic Light Scattering (DLS)	Average particle size, size distribution, PDI.
			Nanoparticle Tracking Analysis (NTA)	Tracks individual particles; gives number-based size distribution.
		Zeta Potential	Electrophoretic Light Scattering	Surface charge; predicts colloidal stability.
		Lipid Composition	High-Performance Liquid Chromatography (HPLC)	Quantifies and identifies lipid components.
2.	Liposome Properties	Encapsulation Efficiency	UV-Visible Spectroscopy / Fluorescence Spectroscopy	Measures amount of drug encapsulated in vesicles.
		Stability Studies	DLS, zeta potential monitoring over time	Tracks size, PDI and charge during storage (temperature, pH).
		Drug Release Kinetics	In vitro release studies	Determines rate and extent of drug release.
3.	Biological Evaluation	In vitro	Cell viability assays (MTT, Alamar Blue)	Cytotoxicity of liposomal formulation.

			Cellular uptake studies	Measures internalization and delivery efficiency.
		In vivo	Animal studies	Pharmacokinetics, biodistribution, therapeutic efficacy.
4.	Biocompatibility / Toxicity Assessment		Hemolysis assay	Measures RBC damage potential.
			Immunogenicity testing	Immune response evaluation.
5.	Drug Release Studies		Dialysis method	Release kinetics under sink conditions.
			Franz diffusion cell	Drug permeation through membranes, often for topical/transdermal.
6.	Surface Modification		X-ray Photoelectron Spectroscopy (XPS)	Analyze liposome surface modifications.
			Fourier-Transform Infrared Spectroscopy (FTIR)	

APPLICATION:[7,22]

1) Cancer chemotherapy.
2) Ophthalmic delivery of drugs.
3) Gene therapy.
4) Topical application.
5) Liposomes as carriers for vaccines.
6) Liposomes as carrier of drug in oral treatment.
7) Cosmetic preparations.
8) Liposomes for pulmonary delivery.
9) Sustained release drug delivery.
10) Intracellular drug delivery.
11) Food industry.
12) Cell biological application.
13) Lysosomal storage disease.
14) Mental storage disease.
15) Transdermal drug delivery.

LIMITATION:(31)

1) Encapsulation efficiency
2) Stability
3) Sterilization
4) Active targeting
5) Gene therapy
6) Lysosomal degradation

NOVEL LIPOSOMES:[26]

Sr. No.	Type	Composition	Key Property	Mechanism	Major Applications
1.	Archaeosomes[32,33]	Lipids derived from archaeal microorganisms containing ether or tetraether linkages	Exceptional membrane stability	Resistant to oxidation, heat, and extreme pH; improved structural integrity	Vaccine delivery, oral and targeted drug delivery

2.	Transferosomes[34]	Phospholipids with edge activators (surfactants) providing membrane flexibility	Highly deformable vesicles	Squeeze through intercellular skin pathways, enhancing transdermal penetration	Transdermal drug delivery, peptides, anti-inflammatory drugs
3.	Ethosomes[35]	Phospholipids with high ethanol concentration	Enhanced skin permeation	Ethanol fluidizes lipid bilayers and increases drug penetration through stratum corneum	Topical, transdermal, cosmetic and dermatological delivery
4.	Stealth Liposomes[36]	Phospholipid vesicles coated with polyethylene glycol (PEG)	Prolonged systemic circulation	PEG coating reduces recognition by reticuloendothelial system, improving half-life	Cancer therapy, long-circulating injectable formulations
5.	Pharmacosomes[37]	Drug–lipid conjugates forming amphiphilic complexes	Improved drug stability and permeation	Covalent linkage enhances membrane interaction and bioavailability	Delivery of poorly soluble drugs
6.	Immunoliposomes[38]	Liposomes conjugated with antibodies or ligands	Target-specific delivery	Antibody binding enables selective targeting of diseased cells	Targeted cancer therapy, site-specific drug delivery
7.	Virosomes[39]	Reconstituted viral envelopes (commonly influenza-derived phospholipids and proteins)	Efficient cellular entry	Viral fusion proteins facilitate intracellular delivery	Vaccines, gene delivery, immunotherapy

MARKETED FORMULATIONS: [40]

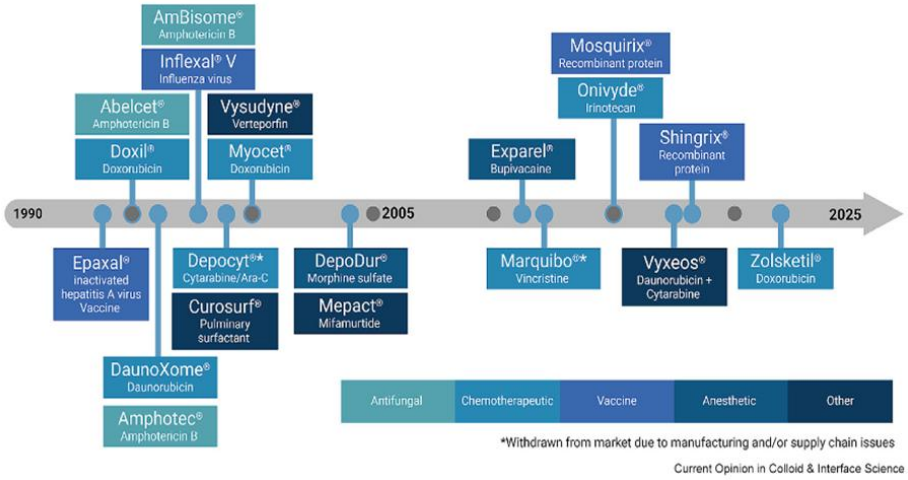


Fig 15: Approval of liposomal therapeutics over the last three decades

CONCLUSION

Liposomes are a well-established and adaptable drug delivery system with proven advantages in enhancing drug stability, bioavailability, and safety. Although challenges such as stability issues, high production cost, and scale-up limitation remain, ongoing advancement in formulation and surface modification have expanded their clinical relevance. With continued research and technological refinement, liposomal systems are expected to play an even greater role in targeted and controlled drug delivery.

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