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Liposomal Expertise: A Comprehensive Exploration of Techniques and Evaluation Parameters for Optimizing Dosage Form

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ABSTRACT

Liposomes, vesicles composed of lipids, have received considerable attention in the scientific community for their effectiveness as drug carriers, particularly in overcoming challenges associated with limited drug permeability. Their bilayer structure, resembling cell membranes, significantly facilitates the movement of active compounds within cells, leading to improved therapeutic efficacy. Liposome formulations featuring vesicles of approximately 100 nm^3 demonstrate notable enhancements in intracellular transport and interactions with cell membranes. Beyond drug delivery, liposomes have diverse applications in medical fields, including antiviral, antifungal, antibacterial, and vaccine medications. They are also utilized in gene therapies, immunology, dermatology, eye diseases, brain targeting, infectious diseases, and cancer therapy, highlighting their potential to revolutionize treatment outcomes across various medical domains. The preparation of liposomes involves both passive and active loading techniques, such as vertexing a thin lipid film with an aqueous solution, reversed-phase evaporation, ethanol injection, ether injection, and sonication. Critical physicochemical attributes, such as particle size and drug encapsulation efficiency, play a crucial role in optimizing liposomal performance. Additionally, evaluating drug release and conducting *in vitro* drug release studies of liposomes are integral components. This comprehensive review explores various methods for liposome preparation, recent studies on excipients used in liposomal formulations, and diverse administration routes to target liposomes for specific diseases. The aim is to contribute to ongoing advancements in the field, fostering innovation and enhancing therapeutic approaches by providing a thorough update on liposomal delivery research.

INTRODUCTION

In past few decades, a number of innovative microparticulate carrier systems like microemulsion, nanoemulsion, nanoparticles, liposomes, ethosomes have been reported for improvement of drug delivery of many potential hydrophilic and hydrophobic drugs. Liposomes are self-assembled phospholipid-based drug vesicles that form a bilayer or a concentric series of multiple bilayers enclosing a central aqueous compartment. The size of liposomes ranges from 30 nm to the micrometer scale.

While significant progress has been made in understanding how physicochemical factors impact the pharmacokinetics of oncology drugs in liposomal formulations, defining drug and carrier pharmacokinetics lacks the precision seen in other therapeutic areas. Consequently, determining the right qualities for liposomal formulations and integrating nanoparticles in therapeutics relies heavily on experience. However, oncology medications in lipid nanocarriers are increasingly advancing to clinical development and licensure. The prolonged plasma half-life and sustained release of liposomal compositions often enhance the therapeutic index by reducing toxicity and occasionally boosting antitumor effectiveness [1]. To date, there has been an increase in the number of FDA-approved liposomal-based treatments, as well as an increase in the number of clinical trials including a wide range of applications in anticancer, antibacterial, and antiviral therapies. More recent advancements have been investigated for optimizing liposomal-based drug delivery systems with more reproducible preparation technique and a broader application to novel modalities, including nucleic acid therapies, CRISPR/Cas9 therapies, and immunotherapies, to meet the ongoing demand for new drugs in clinics [2].

FORMATION OF LIPOSOMES

Dissolved lipid molecules with a hydrophilic head group and a hydrophobic tail self-assemble into bimolecular lipid leaflets when their solubility in the surrounding media is reduced. In contrast to conventional amphiphiles, which have critical micelle concentrations (CMC) of 10^{-2oi}-10^{-4} M, the CMC of bilayer-producing lipids is four to five orders of magnitude lower, implying that these materials have exceptionally poor water solubility. When lipids are exposed to an aqueous environment, their interactions (hydrophilic interactions between polar head groups and Vander Waals interactions between hydrocarbon chains, as well as hydrogen bonding with water molecules) result in the spontaneous formation of closed bilayers within a certain volume of solvent. These constructions could

include one or more concentric membranes ranging in size from 20 nm to a few m in diameter, with a membrane thickness of about 4-5 nm [3].

STRUCTURE AND PROPERTIES OF LIPOSOMES

Choosing the right lipid and preparation method is crucial in developing effective liposomal systems for medication delivery. Liposomes come in three types—small unilamellar vesicles (SUV), large unilamellar vesicles (LUV), or multilamellar vesicles (MLV)—based on their size and structure. Their surface charge can be positive, negative, or neutral, determined by their composition. For instance, lecithin gives a neutral surface charge, while stearylamine and phosphatidic acid contribute to positive and negative charges, respectively. By adjusting lipid content, production techniques, and encapsulated substances, a wide range of liposomal products can be created [4]. Liposomes are particularly promising for antibacterial therapies due to their natural tendency for surface modification. This feature allows the incorporation of ligands like polymers (e.g., PEGylated liposomes) and various molecules such as antibodies, proteins, peptides, and carbohydrates, as illustrated in Figure 1. Surface modifications enhance liposomes' selective targeting (known as ligand-targeted liposomes) and play a vital role in improving both delivery efficiency and therapeutic effectiveness [5].

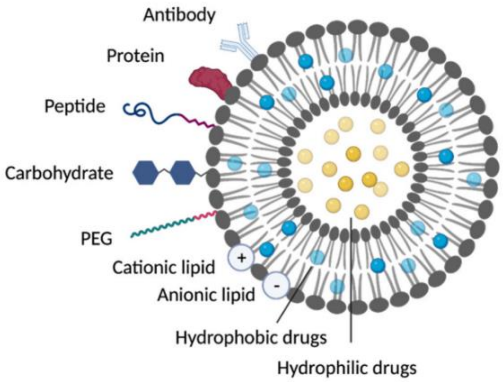

Figure 1: Structure of liposomes

ADVANTAGES OF LIPOSOMAL FORMULATION [6]

- Drug delivery: Liposomes can encapsulate drugs, allowing for targeted delivery and improved therapeutic effects.
- Biocompatibility: Liposomes are biocompatible and can be used to deliver both hydrophilic and hydrophobic drugs.
- Reduced toxicity: Encapsulation by liposomes can help reduce the toxicity of certain drugs to healthy tissues.

- Stability: Liposomes can protect encapsulated drugs from degradation, enhancing their stability and shelf life.
- Versatility: They can be modified to target specific cells or tissues, increasing their specificity, and reducing side effects.
- Immunogenicity: Liposomes have low immunogenicity, reducing the likelihood of adverse immune reactions.
- Combination therapy: Liposomes enable the co-delivery of multiple drugs, allowing for synergistic therapeutic effects.

COMPONENTS REQUIRED FOR FORMULATION OF LIPOSOMES

Phospholipids:

Liposomes mainly contain two categories of phospholipids: glycerophospholipids and sphingomyelins. Glycerophospholipids, found predominantly in eukaryotic cells, have glycerol as their backbone and consist of a hydrophilic head group and a hydrophobic side chain. Different variations in the head group and nonpolar moiety length lead to various glycerophospholipids. Sphingomyelins, crucial for animal cell membranes, have sphingosine as their backbone and typically feature longer acyl lengths than the paraffin residues of sphingosine, making them asymmetric molecules. In liposomal formulation, sphingomyelins have shown good entrapment efficiency, greater serum stability, and a rapid release profile compared to DSPC liposomes. Furthermore, research has demonstrated the successful preparation of small, spherically-shaped vesicles from sphingomyelin solution using ultrasonic–supercritical CO2 technique, although an increase in operating temperature led to aggregation and a larger size distribution of the vesicles [7].

Bilayer Excipients:

Preparing liposomes with just phospholipids is usually not enough, which can lead to issues with the packing of the bilayer and result in drug leakage during storage, especially due to factors like unsaturated fatty alkyl chains or a low phase transition temperature. To prevent this leakage, additional bilayer components such as cholesterol and α-tocopherol are commonly added. Changes in the composition of the phospholipid bilayer can affect how effectively liposomes encapsulate drugs. Cholesterol is used to stabilize aggregates created by electrostatic effects and improve the flexibility of the phospholipid bilayers. The amount of

cholesterol used in liposome preparation varies depending on the intended use of the liposomes. On the other hand, α-tocopherol enhances the therapeutic potential of liposomes by creating reactive oxygen species in damaged tissue, aiding in intracellular delivery, and extending the time drugs encapsulated within the liposomes remain active [8].

Additional Excipients:

Incorporating Polyethylene glycol (PEG) onto the surface of liposomes extends their circulation duration, shields the enclosed drug from deactivation or metabolic breakdown, enhances overall stability, and facilitates improved cellular uptake. PEG enables the development of 'stealth' liposomes, evading easy recognition by the body's immune clearance system. Additionally, PEG mitigates particle aggregation and sustains stability during storage. The efficacy of PEGylated liposomes in entering cells can be heightened by attaching targeting molecules, such as antibodies, vitamins, proteins, and nucleic acids, that specifically recognize and bind to target cells. Advancements in liposomal technologies have progressed to include "second-generation liposomes," specialized vesicles with prolonged circulation in the bloodstream and a controlled, gradual release of pharmaceutical contents. This is achieved through adjustments to the composition, size, and charge of the vesicles' phospholipid membrane. Various particles, such as sialic acid or glycolipids, unmodified dextran’s, and specially modified dextran’s, are employed to craft these modified surface liposomes [9].

Classification of liposomes and method of preparation.

Traditional method of preparing liposomes.

Liposome preparation can be quite versatile and can involve variations and additional steps depending on the intended application and desired characteristics of the liposomes. Various methods are used to make liposomes, and the main ones are explained in Table 1. Liposomes fall into four types based on layers and size:

Table 1

Method of preparation	Type of liposome	Size (nm)
Vertexing thin lipid film with aqueous solution	MLV	400–5000
Sonication	SUV	25–50
French press	SUV	30–50
Ethanol injection	SUV	30–110
Detergent dialysis	LUV	100–200
Ether injection	LUV	150–250
Freeze and thaw	LUV	50–500
Reversed-phase evaporation	LUV	200–900
GUV	GUV	5000–50,000

Multilamellar vesicles (MLVs) with large sizes, small uniflagellar vesicles (SUVs or liposomes) with small sizes, large unilamellar vesicles (LUVs) with large sizes, and giant unilamellar vesicles (GUVs) with a 45 mm diameter. Lipid vesicles (MLVs) with a 4400 nm diameter and water-containing layers display various shapes (spherical, onion-like, oblong, tubular) based on adjusted water content. MLVs are primarily used for studying lipid organization in membranes. Small uniflagellar vesicles (SUVs), 20-50 nm in size, can be directly derived from MLVs through methods like sonication, French press passage, or ethanol injection [10]. SUVs have a 1:2 lipid molecule ratio between inner and outer layers, distinguishing them from large unilamellar vesicles (LUVs) with diameters of 50-200 nm. LUVs are prepared using techniques like detergent dilution, ether injection, freezing and thawing, or reverse-phase evaporation. The behavior of SUV and LUV layers differs due to packing constraints in membranes with large curvature. Giant unilamellar vesicles (GUVs), used as cell models, require 10–20% charged lipids (e.g., PS, PG, or CL) in PC layers for successful preparation at physiological ionic strength. LUVs and GUVs offer advantages in stability, trapped volumes, and trapping efficiencies, with liposome characteristics varying based on composition, particle sizes, membrane fluidity, charge of the membrane surface, and the method of liposome preparation [11].

Vertexing thin lipid film with aqueous solution:

The process of preparing Multilamellar Vesicles (MLVs) started with evaporating 100 mg of Egg Phosphatidylcholine (EPC) in chloroform in a 50-mL round-bottom flask using a rotary evaporator at room temperature. A 2-milliliter aqueous phase, typically a HEPES buffer, was then added to the flask, along with any intended solute for entrapment. The contents of the flask were vortexed until complete suspension of the lipid film, followed by a 2-hour equilibration period on the bench and four subsequent wash cycles.During each wash cycle, the liposome suspension was mixed with buffer to reach a total volume of 20 mL. Subsequently, centrifugation at 10,000g was performed to pellet the liposomes, with careful removal of the supernatant. The resulting pellet underwent resuspension for further washes or to reach a specified final volume. This approach reliably ensured the preparation of MLVs tailored to the desired lipid composition and entrapment characteristics [12]. (Sol M. Gruner.,*et.al* 1984)

Sonication method:

The sonication method for preparing liposomes involves several key steps. First, a lipid solution is evaporated to create a thin lipid film in a round-bottom flask or glass vial using vacuum or nitrogen gas. Next, an aqueous solution, like a buffer or distilled water, is added to hydrate the lipid film. The hydrated lipid mixture is then subjected to sonication, exposing it to high-frequency sound waves (ultrasound) to break down lipid aggregates and form smaller vesicles. The duration and intensity of sonication determine whether small or large uniflagellar vesicles are obtained. The resulting liposome dispersion is analyzed for size, polydispersity, and zeta potential using techniques like dynamic light scattering and zeta potential analysis. Sonication is widely valued for producing consistently sized liposomes, playing a crucial role in drug delivery and various applications [13]. (Chandraprakash Dwivedi., 2014).

Ethanol injection:

The significance of the ethanol injection technique in the pharmaceutical industry lies in its role in scaling up processes. Numerous scientists have extensively adapted and documented modifications in the literature to enhance liposome productivity, refine physical properties, ensure uniformity, and establish a convenient and scalable industrial method. In contrast to the traditional approach, where an ethanolic solution containing drugs and lipids is injected into the aqueous phase, this method generates encapsulated vesicles without the need for an

external energy source. To ensure homogeneity in small unilamellar vesicles (SUVs), the ethanol concentration should not surpass 7.5% of the total formulation volume. The use of a dialysis membrane efficiently separates residual ethanol, leaving a pure aqueous solution, and sample concentration can be achieved using a filtration tube under nitrogen gas pressure [14]. (Ahmed Gouda.,2020)

Ether injection:

A solution of lipids is mixed with either an ether-ethanol mixture or diethyl ether. This mixture is then slowly injected through a thin needle into an aqueous solution containing the material to be encapsulated. This process takes place either at the temperature at which the organic solvent vaporizes or under reduced pressure, resulting in the creation of liposomes. When the compound to be encapsulated is exposed to higher temperatures, its degradation occurs, but this can be mitigated by using fluorinated hydrocarbons (Ferons) instead of ether. While the volume encapsulated per mole of lipid remains high at 8-17/mol, the efficiency of the formed liposomes is relatively low [15] (Akbarzadeh et al., 2013).

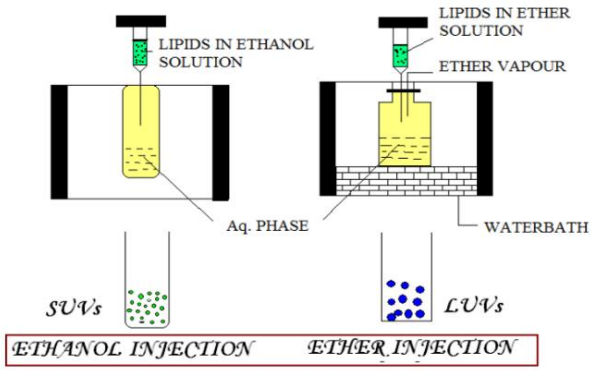

Figure 2: Method of preparation of Liposomes by solvent dispersion

Reverse Phase Evaporation:

The Reverse Phase Evaporation Technique is a systematic process for crafting liposomes with specific attributes. Initially, an organic phase is established by introducing a lipid mixture into a flask. Subsequently, solvents are evaporated, leading to the creation of lipid films. These films are then dissolved in an organic phase, primarily comprised of isopropyl ether and/or diethyl ether. Following this, the water phase is introduced, establishing a two-phase system. Sonication is applied for uniform dispersion, and as the organic solvent

gradually evaporates, the system transforms into a viscous gel, resulting in the development of a liposome-containing aqueous suspension. Notably, compared to the thin-film hydration method, the reverse phase evaporation technique excels in achieving higher internal aqueous loading. Optional steps may include employing techniques like dialysis and centrifugation to remove any residual solvent, with awareness that a small amount of organic solvent may persist and potentially interact with lipids or drugs [16].(Pradnya Palekar-Shanbhag.,2020).

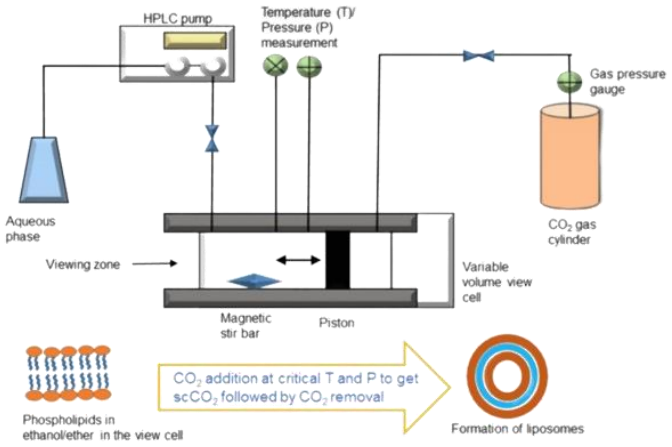

Figure 3: Reverse Phase Evaporation

French press:

The process of generating liposomes through high-pressure techniques involves the application of increased pressure, leading to the development of liposomes, either uniflagellar or oligolamellar, with sizes ranging from 30 to 80 nm. These liposomes demonstrate improved stability compared to those formed by sonication. However, this method has its drawbacks, including the initial cost for the high-pressure equipment and pressure cell. Nevertheless, liposomes prepared using this technique exhibit fewer structural defects than those formed through sonication [17].

Innovative methods for liposome preparation.

Double solvent displacement method (DSD):

The DSD (solvent displacement method) is a novel approach to creating lipid nanocarriers, allowing to produce liposomes and solid lipid nanoparticles (SLNs) with adaptable. operational conditions. This method primarily utilizes phospholipids and is focused on encapsulating cyclosporine, an immunosuppressive cyclic polypeptide. The process commences with the initial solvent displacement, which triggers the formation of phospholipid nanoparticles in a free-water medium, resulting in the creation of self-organized

structures known as supra micelles. Subsequently, the introduction of an aqueous medium prompts the formation of liposomes or SLNs. During the first phase, cyclosporine is mixed with phospholipids in ethanol, and the concentration of ethanol plays a crucial role in determining whether liposomes or SLNs are produced. Characterization of the resulting nanostructures, conducted with a phospholipid concentration of 8.5 mg/ml, demonstrates successful liposome formation with 16.6% ethanol and SLN generation with up to 10% ethanol. Both populations exhibit nanosized and homogeneous features, with liposomes at 107 nm (PI: 0.24) and SLNs at 96 nm (PI: 0.25). The encapsulation efficiency falls between 65% and 75%. This innovative method involves two critical steps, emphasizing the organizational modifications of phospholipids. The first step, characterized by an intermediate state and self-assembly into supra micelles, is considered a pivotal stage, showcasing the originality and innovation of the DSD method. Significantly, existing research does not mention a technique that enables the encapsulation of a polypeptide such as cyclosporine into liposomes or SLNs through simple adjustments in operational conditions [18].

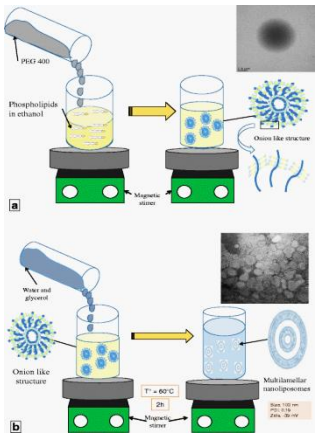

Figure 4: Schematic illustration of the first (a) and second (b) steps of the lipid vesicles formation by double solvent displacement.

Supercritical fluids (SCFs) methods for liposome preparation:

Traditional methods for making liposomes, like thin film hydration or ethanol injection, have downsides such as uneven distribution, instability, excessive solvents, and potential side effects. The use of organic solvents in these methods can harm the environment and human health due to toxicity and ingredient degradation, especially for protein drugs [19]. New techniques involving supercritical fluids, like Supercritical Anti-Solvent (SAS), Depressurization of an Expanded Liquid Organic Solution Suspension (DELOS), and Supercritical Assisted Liposome Formation (SuperLip), are being developed as eco-friendly

alternatives to traditional liposome production [20]. These methods are considered environmentally friendly or "green technologies." Carbon dioxide is widely used due to its safety, cost-effectiveness, and easily manageable critical parameters. Liposomes produced using supercritical CO2 exhibit improved integrity, sphericity, and uniformity compared to those made through thin film hydration. Dense phase CO2 transforms phospholipid aggregates into nano/microparticles, with characteristics adjusted by processing parameters. The resulting liposome size is controlled by the rate of decompression and the opening diameter of the nozzle [21].

a) Supercritical anti-solvent (SAS): Lipid vesicles, known as MLVs, exhibit diverse shapes such as spherical, onion-like, oblong, and tubular, with a diameter of 4400 nm. These vesicles are crucial for studying how lipids organize in membranes. From MLVs, small unilamellar vesicles (SUVs) of 20-50 nm can be directly produced through methods like sonication or ethanol injection, featuring a distinct 1:2 lipid molecule ratio between inner and outer layers. In contrast, large uniflagellar vesicles (LUVs) with diameters of 50-200 nm are prepared using techniques like detergent dilution or reverse-phase evaporation. The behavior of SUV and LUV layers differs due to membrane curvature constraints. Giant unilamellar vesicles (GUVs), used as cell models, require 10–20% charged lipids for successful preparation, offering advantages in stability and trapping efficiencies. Liposome characteristics vary based on composition, particle sizes, membrane fluidity, charge of the membrane surface, and the method of liposome preparation [22].

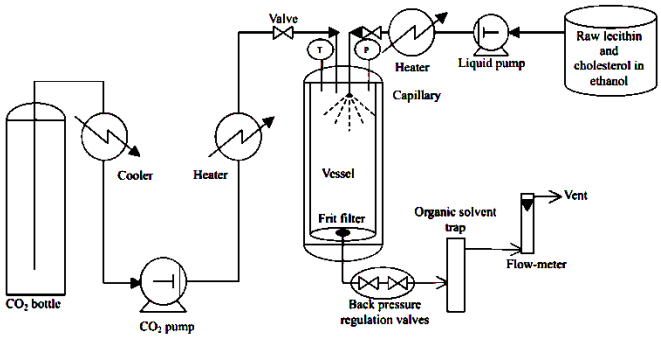

Figure 5: Experimental set-up for the SAS process

b) Depressurization of an expanded liquid organic solution-suspension (DELOS) method:

The DELOS (Depressurization of an Expanded Liquid Organic Solution) crystallization technique efficiently produces submicron or micron-sized crystalline particles in a single step. It works by rapidly and uniformly lowering the temperature in an organic solution,

which is expanded with a gas-like compressed fluid like CO2 during depressurization. The process starts with phospholipid (PL) molecules in bilayers or organized curvature in an aqueous medium at room temperature. During pressurization in the second stage, CO2 quickly dissolves in the aqueous phase, leading to a dissociation equilibrium. In the third stage, depressurization swiftly releases CO2 from PL bilayers, temporarily breaking them into highly dispersed Pl. In the fourth stage, after an instantaneous solution forms, temporarily separated PLs and cholesterol (CH) rapidly reorganize, forming drug-loaded liposomes. The DELOS process is proven to generate micron-sized solid particles, with characteristics influenced by the initial supersaturation ratio and solution composition before depressurization. Importantly, the working pressure in DELOS is moderate compared to other high-pressure crystallization techniques, potentially making it economically viable for industrial use [23].

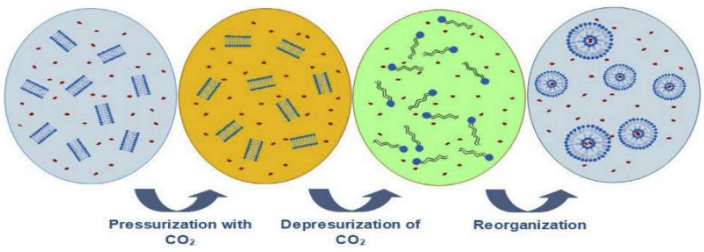

Figure 6: Liposome formation by the DELOS method

Heating methods:

Nkanga, Krause, and their team devised an efficient method for crafting liposomes containing isoniazid-conjugated phthalocyanin cyclodextrin complexes. This technique, employing a solvent-free and easily scalable heating approach, involves hydrating agents like ethylene glycol, propylene glycol, or glycerol. The phospholipid and cyclodextrin-drug complex undergoes 60 minutes of hydration at room temperature with water. Afterward, the adjuvant is introduced, and stirring continues for an additional hour at 70°C, leading to the self-assembly of phospholipids into liposomes encapsulating the complex. The reported entrapment efficiency ranges from 58% to 70%, with a particle size between 150 and 650 nm. Interestingly, entrapment efficiency remains consistent across different hydrating adjuvants, and surprisingly, liposomes without the adjuvant exhibit higher efficiency at 71%. In comparison to the film hydration method, the heating method without adjuvant results in increased efficiency and size. Additionally, Basiri et al. adapted a heating method by Mozaffari et al. to create surfactant vesicles encapsulating alpha-tocopherol. This process,

aligned with green chemistry principles, supports scalable applications for encapsulating both hydrophilic and lipophilic drugs [24].

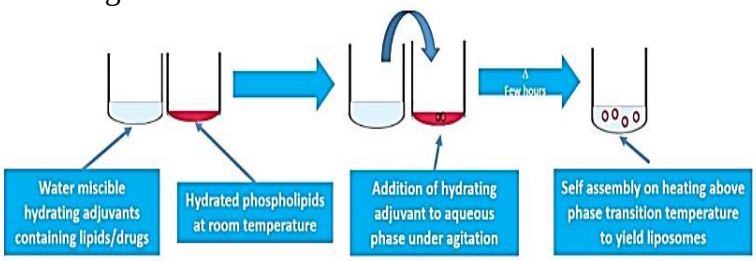

Figure 7: Schematic representation of heating process to fabricate liposomes.

Freeze drying of double emulsions: The method involves freeze-drying lipids and water-soluble carriers in a co-solvent system of tert-butyl alcohol and water. This results in cakes containing a uniform solution. Adding water transforms the freeze-dried product into a consistent dispersion of multilamellar vesicles (MLVs) that can be resized through extrusion [24]. However, freeze-drying faces a challenge due to the low encapsulation efficiency of liposomes. To address this, Wang and colleagues introduced an innovative approach: freeze-drying a double emulsion W1/O/W2, where W1 and W2 are aqueous phases, and O is the organic phase containing dissolved phospholipid. The resulting liposomes, measuring below 200 nm, show varying encapsulation efficiency with different substances; for example, calcein exhibits 87% efficiency, while 5-fluorouracil shows only 19%. Freeze-drying water-in-oil emulsions with phospholipids produces lyophiles, which, upon rehydration, generate liposomes under 200 nm with encapsulation efficiency exceeding 60% for three different drugs. This freeze-drying technique addresses stability concerns in liposomes with heat-sensitive products, involving the removal of water from frozen liposomal products at extremely low pressures in the presence of sugars (sucrose, trehalose) to prevent leakage of encapsulated materials and prevent an increase in liposome size during rehydration [25].

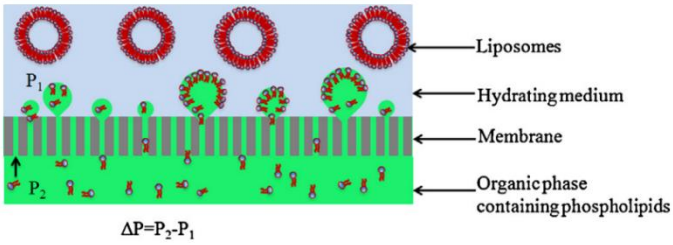

Figure 8: Liposome formation in a membrane contactor

Influential factors in drug release from liposomes: Liposomes are successful systems for delivering tiny drug particles precisely where they are needed. The field of liposomal drug

delivery has made significant progress, resulting in approved formulations known for their effectiveness and safety. For these drugs in liposomes to work well, it is important to control how they are released [26]. Different methods in the literature focus on improving and optimizing this release rate. Factors like the amount of cholesterol in the liposomes, the type of drug inside, the liposome's membrane composition, and external conditions can all influence how the drug is released [27].

Evaluation test for liposomes

a) Entrapment Efficiency

Ultra-filtration, dialysis, small-angle X-ray scattering, and chromatography techniques can all be used to determine the entrapment efficiency and drug loading of liposomes. Fluorescence correlation spectroscopy, HPLC analysis, and a UV spectrophotometer can all be used to further analyze the amount of medication that is not entrapped. The concentration of unentrapped drugs is calculated and deducted from the overall amount of drug added in the formulation. The drug amount is then analyzed using radioactivity or a spectrophotometer [31].

$$EE = \frac{Amount\ of\ entrapped\ drug}{total\ amount} * 100$$

b) Number of lamellae:

The number of lamellae can be determined using a variety of methods, including electron microscopy, AFM, NMR, and small-angle X-ray spectroscopy. In situ energy-dispersive x-ray diffraction combined with small-angle X-ray scattering can be utilized to quantify the thickness of bilayers [32].

c) Vesicle morphology:

The method used for morphological analysis was Transmission electron microscopy. Through vitrification of the samples in a thin layer suspended between grids covered with polymer, cryogenic-TEM enables direct viewing of samples in their hydrated condition. Due to the difficulties associated with dehydration, traditional (negative staining) transmission electron microscopy (TEM), which involves drying objects on carbon grids before viewing them under a microscope, is not advised. With its ability to directly visualize and validate crystalline symmetry, cryo-TEM is a potent addition to scatter data. Cryo-TEM combined with scattering is the gold standard for characterizing the structure type of non-lamellar liquid-crystalline dispersions [33].

d) pH-sensitivity, of liposomes

Fluorescence was measured using a Perkin Elmer LS5 spectrofluorometer with excitation wavelength (λc) set at 490 nm and emission wavelength at 520 nm. Liposomes (10 µl) were introduced into 1.99 ml PBS at the desired pH and incubated at room temperature for a specified duration. After incubation, the mixture's pH was readjusted to 8.0 by adding an appropriate amount of NaOH solution. Subsequently, deoxycholate (final concentration 0.25%) was added to fully release calcian from the liposomes [34]. The percentage of liposome leakage was determined based on the reduction in calcein fluorescence using the formula.

Where: Percent Leakage $= \left(\frac{F_0 - F_f}{F_t - F_t}\right) \times 100$

- F_0 is the fluorescence intensity of liposomes in PBS at pH 8.0 before incubation.
- Ff is the fluorescence intensity of liposomes after incubation.
- Ft is the total fluorescence intensity of liposomes incubated at pH 8.0 after deoxycholate addition.
- Ft' is the total fluorescence intensity of liposomes incubated at different ph. $\frac{F_t}{F_t'}$ was used to normalize the number of liposomes in the measurement [35].

***In vitro* release of drugs from liposomes:**

The dialysis membranes were soaked overnight to ensure full saturation. Subsequently, two milliliters of liposomes containing the specific drug or the free drug were placed in a dialysis bag, which was then transferred into a larger quantity of the same solution. Stirring took place both inside and outside the bag using a magnetic stirrer and an electric stirrer, respectively. At regular intervals, 200 ml samples were extracted from outside the bag, and an equal amount of solution was added back. These samples underwent analysis for budesonide and hydrocortisone using High-Performance Liquid Chromatography (HPLC). The HPLC setup featured a Beckman solvent module, a Beckman UV detector set at 215 nm, a System Gold data module, a Marathon autosampler equipped with a column thermostat, and a Rheodyne injection valve with a 20 ml loop. Hydrocortisone and budesonide were analyzed using an LC-8 column, with the mobile phase consisting of a mixture of acetonitrile and water. The flow rate was 1.0 ml/min, and the quantitation limit was 0.1 mg/ml. The dose-response curve demonstrated linearity within the concentration range of 0.1 mg/ml to 1.05 mg/ml, with a high level of reproducibility (6.2% RSD) for repeated assays of a budesonide standard solution at 0.1 mg/ml [36].

Conclusion:

The adaptability and effectiveness of liposomes as drug delivery systems across various drug types and administration routes are evident. Despite advancements in production technology and FDA approval, there is an ongoing need to meet clinical requirements. Understanding the importance of each structural element in developing stable and effective liposomal drug formulations is crucial. Future research should prioritize evaluating the biocompatibility, circulation rate, and toxicity of potential drug-loaded liposomes to streamline the drug development process. The advantages of combination drug therapy and innovative drug products using liposomal formulations underscore the prominence of liposomes as a preferred drug delivery strategy. However, it is important to note that while liposome technology is valuable, it may not be universally suitable for all drugs. Researchers are actively exploring the technical boundaries of liposomes, especially in predicting drug/lipid interactions for rational design, as the development of liposomal formulations still relies predominantly on trial and error.

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