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A DYNAMIC EVALUATION OF LIPOSOME: A NEW DRUG DELIVERY SYSTEM USING THEIR METHODOLOGIES

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ABSTRACT

'Liposomes' distinct structure and adaptability as an encapsulating medicinal agent have made them a popular medication delivery method. Their unique composition and versatility in encasing therapeutic agents have rendered them a widely used mode of pharmaceutical administration. This thorough analysis highlights the relevance of liposomes in pharmaceutical research and development by examining the many approaches used in their production. Liposomes are spherical vesicles composed of lipid bilayers used in targeted delivery, controlled medication release, biocompatibility, thin-film hydration, reverse-phase evaporation, and other significant technological methods. More recently, microfluidics has been a popular methodology. Furthermore, advancements in surface modification strategies are marked, highlighting their role in enhancing liposomal stability, targeting specific tissues, and prolonging circulation times in vivo. This review provides insights into optimizing drug delivery systems for improved therapeutic outcomes by elucidating the methodologies and advancements in liposome preparation.

Keywords: - Liposomes, Drug delivery, Encapsulation, Microfluidics, Surface modification

- ## OVERVIEW OF NOVEL DRUG DELIVERY SYSTEM

A wide range of complex techniques, formulations, technologies, and systems known as Novel Drug Delivery Systems (NDDS) are used to safely and precisely distribute pharmaceutical chemicals inside the body to provide the intended therapeutic effects. NDDS is a system that is used for drug delivery in ways that go beyond standard approaches. It combines cutting-edge procedures with creative dose forms that work better than traditional ones. This system beyond conventional methods, the NDDS system is utilized for drug delivery. It blends innovative dosage formulations with state-of-the-art methods that outperform conventional ones. Some merits of innovative drug delivery systems include optimal dosage at the appropriate time and place; effective use of costly medications; lower manufacturing costs; advantages for patients; and increased comfort

and level of living. Through the use of innovative technologies and materials, these systems seek to maximize treatment efficacy, reduce adverse effects, and increase patient compliance.[1]

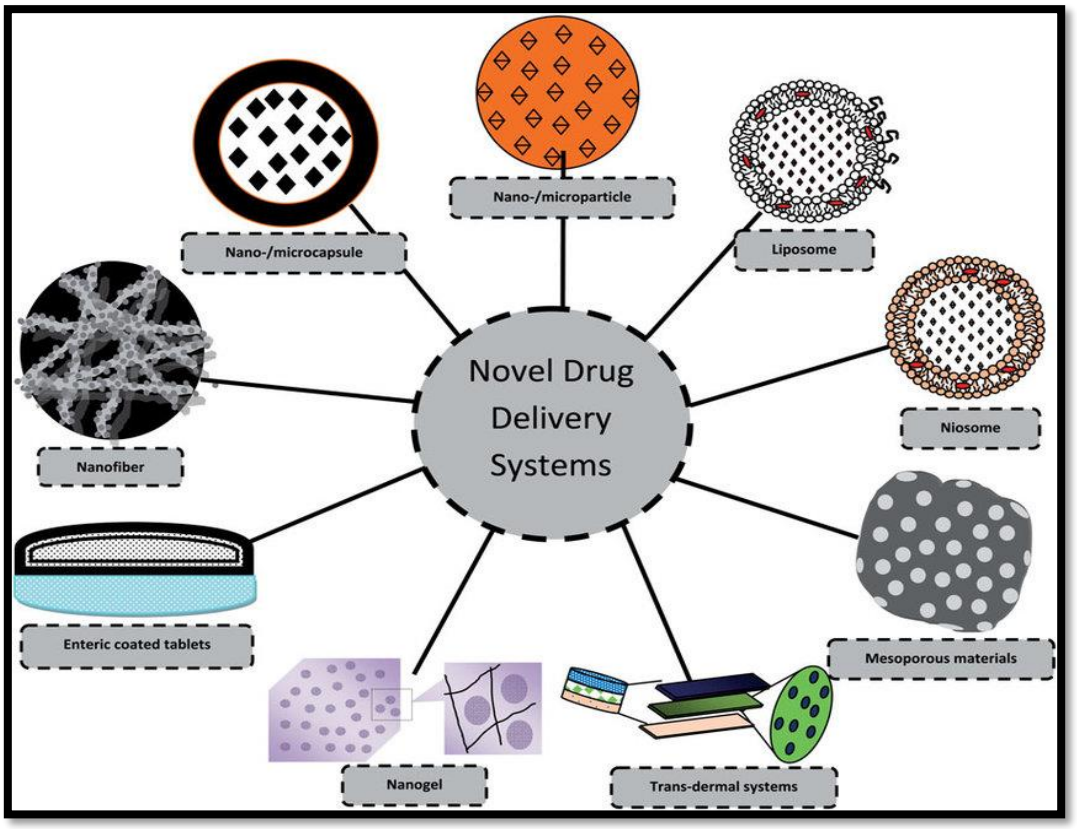


Figure 1: Pictorial representation of NDDSs[2]

- **Strength of Novel Drug Delivery Systems[3,4]**

1. **Targeted Delivery**: Drugs may now be delivered precisely to the site of action thanks to NDDS, which reduces exposure to the systemic environment and harmful effects on healthy tissues.
2. **Enhanced Therapeutic Efficacy**: By optimizing medication release profiles and enhancing bioavailability, NDDS enhance the therapeutic efficacy of drugs, potentially allowing for lower doses and reduced dosing frequency.
3. **Improved Patient Compliance**: The controlled and targeted delivery offered by NDDS can improve patient adherence to treatment regimens, leading to better clinical outcomes.
4. **Cost Efficiency**: NDDS can contribute to cost savings by maximizing the therapeutic benefits of drugs, reducing the amount of active ingredients required per dose, and minimizing waste.

5. **Innovative Dosage Forms**: These systems introduce novel dosage forms such as nanoparticles, liposomes, implants, and microparticles, which offer advantages in terms of stability, shelf life, and ease of administration.
6. **Personalized Medicine**: NDDS technologies support the concept of personalized treatment by adjusting medication administration to meet the needs of each patient, thereby optimizing treatment outcomes.

- **LIPOSOMES[5,6]**
- **INTRODUCTION**

The term "liposome" derives from the Greek words "lipo" (-stands for fat) and "soma" (-stands for body or internal fat). Liposomes are artificial spherical nanostructures composed of one or more phospholipid bilayer membranes. Liposomes are tiny, colloidal, bilayered vesicles with an aqueous core surrounded by a naturally occurring or synthetic lipid bilayer. The ingredients are phosphatidylcholine (lecithin), phosphatidylethanolamine, phosphatidylglycerol, and phosphatidylserine. These lipids are amphiphilic, meaning they have a lipophilic tail and a cluster of polar heads, which makes them ideal for creating liposomes. The aqueous core of the liposome is surrounded by a phospholipid bilayer. The core contains the drugs, whereas the hydrophobic domain contains the water-soluble ones.

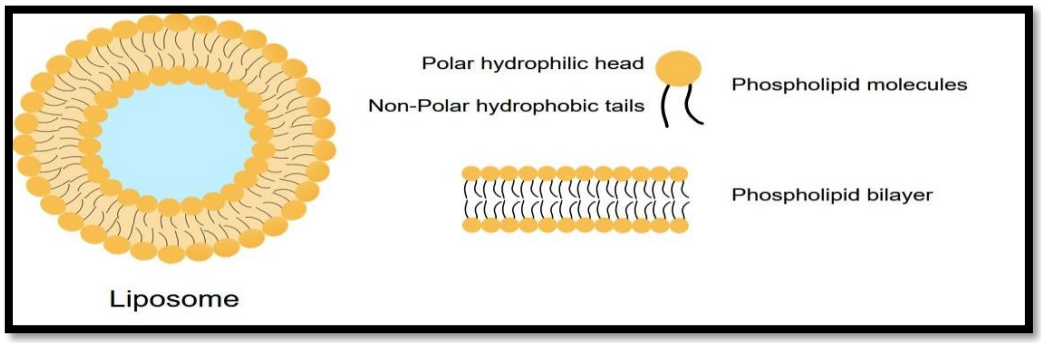


Figure 2: Diagrammatic illustration of the liposome's structure.[6]

This kind of spherical lipid vesicles can contain one or more phospholipid bilayers; polar medicinal molecules can be encapsulated due to the polar nature of the liposomal core; amphiphilic and lipophilic compounds become soluble within the phospholipid bilayer based on their affinity for the phospholipids; niosomes are generated when non-ionic surfactants, instead of phospholipids, are involved in the bilayer-building process; the size of liposomes varies from 20 nm to 1000 nm, depending on the number of layers. Since their structural similarities to cell membranes (lipid bilayer), liposomes are the most extensively utilized active substance delivery vehicles. They have a prime encapsulation capacity, therapeutic index, biocompatibility, biodegradability, flexibility, and safety. Since the therapeutic impact of the encapsulated substances is obtained by minimizing the dose and frequency of administration, the liposome-based delivery method is widely used in the pharmaceutical industry. Numerous studies have been conducted on liposomes as delivery systems for proteins, nucleic acids, imaging agents, and small molecules. To increase therapeutic effectiveness, several delivery methods, including pulmonary, oral, transdermal, parenteral, ocular, and nasal routes, have been devised.

Liposomes are used in medicine to deliver gene treatments, vaccinations, and chemotherapeutic medications. By concentrating on certain tissues, they can prevent medications from breaking down in the body and lessen unwanted effects. Formulations containing liposomes have been produced to treat fungus infections, cancer, and even hereditary diseases. The pharmaceutical and nutraceutical sectors are served by a wide variety of liposomal products available on the market. Examples include VENTUS liposomal Omega 3, LVC5, Liposomal Vegan D3 K2 Magnesium, and other supplements. Similar to this, a large variety of commercial Nano-liposomal products are offered by the cosmetics industry. The high-esterified essential fatty acid concentration of the phospholipid component of liposomes makes them very intriguing.[7]

Although phosphatidylcholine has conditioning and softening qualities, it is preferred as a necessary ingredient for creating liposome membranes. Liposomes' lengthy and gradual dermal releases, along with their skin-friendly nature, make them suitable for delivering active substances. Liposomes safeguard and regulate the release of sensitive active ingredients in dietary supplement formulations.

- **EVOLUTION OF LIPOSOMES (1960- PRESENT):[8,9,10]**

Liposomes are microscopic vesicles composed of phospholipid bilayers and were initially identified in the early 1960s by British scientist Alec D. Bangham. Working at the Babraham Institute near Cambridge, Bangham was investigating the structure of biological membranes. His experiments involved studying the behavior of phospholipids, particularly phosphatidylcholine from egg yolks, in aqueous solutions.

Bangham noted that when phospholipids were combined with water, they spontaneously formed spherical structures with a lipid bilayer membrane—a structure remarkably similar to the lipid bilayers found in cell membranes. These spherical structures encapsulated a small volume of aqueous solution inside the phospholipid membrane. These formations were first described by Bangham as "small fat bubbles."

Later, Bangham and his colleagues, including Gerald Weissmann, began to recognize the potential applications of these lipid vesicles. Weissmann, in particular, coined the term "liposome" to describe these structures, combining the Greek words "lipos" (fat) and "soma" (body). This term reflected their lipid composition and vesicular nature. An important advance in biology and medicine was the discovery of liposomes. They provide a cutting-edge resource for researching the composition and operation of membranes. Furthermore, they were attractive candidates for drug delivery systems due to their propensity to encapsulate hydrophilic and lipophilic molecules in their lipid bilayers and aqueous compartments. Since their discovery, liposomes have been extensively studied and developed for several applications in medicine, including vaccine delivery, gene therapy, and targeted drug delivery. They have also found applications in cosmetic formulations and food industries.

1. **1960s-1970s:** Liposuction was pioneered in the late 1960s by European surgeons, initially using blunt cannulas and high-vacuum suction to remove fat deposits. This

technique, known as "dry liposuction," was associated with higher risks of bleeding and tissue damage.

2. **1980s-1990s:** The introduction of the tumescent technique in the 1980s revolutionized liposuction by injecting a solution (tumescent fluid) into the targeted fat areas before suction. This fluid contained a local anesthetic and epinephrine, which minimized bleeding and improved patient comfort.
3. **2000s-2010s:** The development of ultrasound-assisted liposuction (UAL) and laser-assisted liposuction (LAL) in the 2000s allowed for improved skin tightening and more precise fat removal. UAL employs ultrasonic energy to liquefy fat cells, whereas LAL uses laser energy for the same purpose.
4. **2010s-Present:** With the invention of techniques like power-assisted liposuction (PAL) and water-assisted liposuction (WAL), liposuction technologies are still evolving. WAL utilizes a water jet stream to loosen and remove fat cells, whereas PAL adds mechanical motion to improve fat removal.

- **Since the 1990s, liposomes have also been utilized for clinical and commercial purposes.[11]**

1. In the 1990s, several liposomal formulations received regulatory approval and entered clinical use.
2. Doxil® (pegylated liposomal doxorubicin) was one of the first liposomal drugs approved by the FDA (Food Drug Administration) in 1995 for the treatment of ovarian cancer.
3. Liposomal amphotericin B became a standard treatment for fungal infections, offering improved safety and efficacy compared to conventional formulations.
4. The commercial success of these products spurred further research and development into advanced liposomal technologies.

- **LIPOSOME CLASSIFICATION:[12]**

Liposomes are divided into several types according to their size and lamellarity: giant unilamellar vesicles (>1000 nm), small unilamellar vesicles (20–100 nm), oligolamellar vesicles (100–1000 nm), and large unilamellar vesicles (>100 nm), multilamellar large vesicles (>500 nm), and multivesicular vesicles (>1000 nm). The encapsulation efficiency of hydrophilic substances in liposomes decreases with fewer bilayers and it increases with larger liposome size. Size is a critical factor influencing the half-life of liposome circulation.

The types and characteristics of liposomes affect the encapsulation of drugs. Typically, liposomes used for drug delivery range from 50 nm to 150 nm in size. Various theories describe the interaction of liposomes with cell membranes, including local fusion (adhesion), specific absorbance into the cell membrane, phagocytosis, and nonspecific (receptor-mediated) endocytosis. Factors influencing liposome-cell interactions include composition, surface charge, liposome size, biological environment, and the presence of targeting ligands on the liposome surface.

1. **SUVs, or small unilamellar vesicles: -**[13] it is composed of a monophospholipid bilayer in a unilamellar form. SUVs are made up of an aqueous core surrounded by a single lipid bilayer. They are tiny in size compared to MLVs. SUVs are often used for targeted drug delivery due to their ability to penetrate cellular more efficiently.

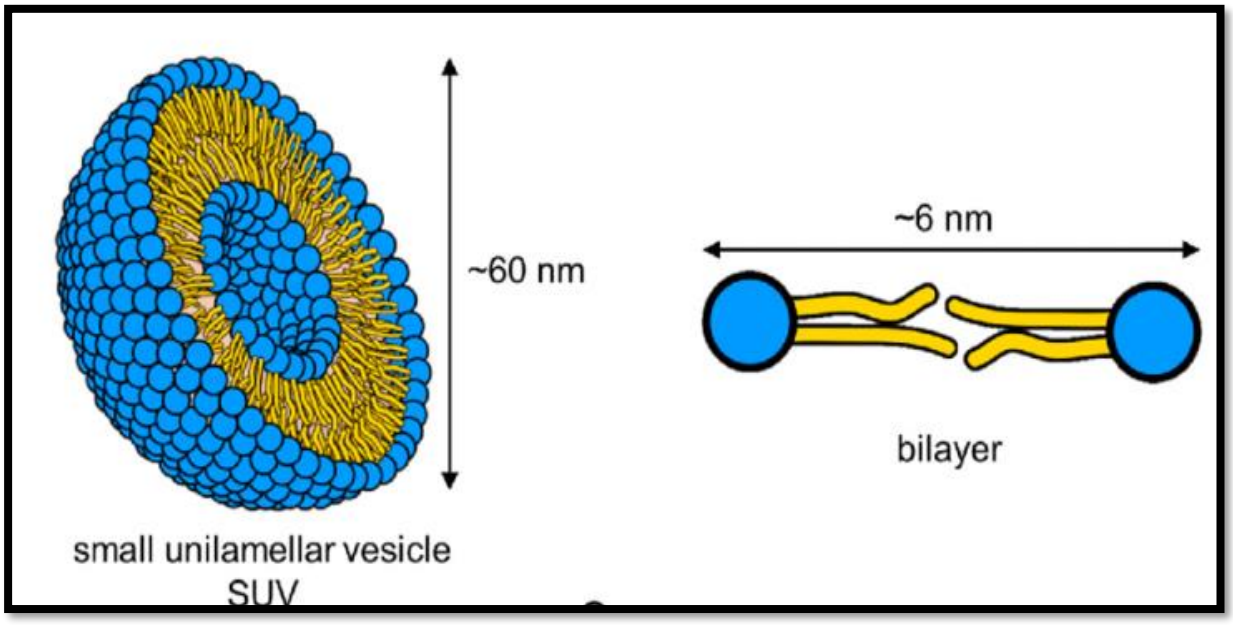


Figure 3: Diagrammatic representation of small unilamellar vesicles (SUVs)[14]

2. **Vesicles with multiple lumens (MLVs):** Multiple lipid bilayers are assembled into MLVs and are spaced apart by aqueous layers. When compared to other kinds of liposomes, they are bigger. MLVs are useful for encapsulating hydrophobic and hydrophilic drugs and sustained release profiles are provided.

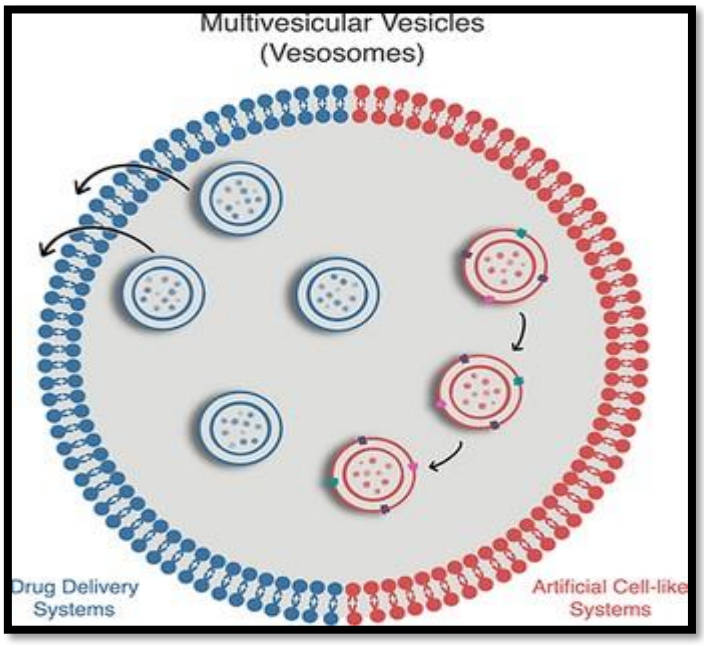


Figure 4: Diagrammatic -representation-of-multi-lamellar-vesicles-SUVs[15]

3. **Huge Vesicles (Large Unilamellar):** LUVs are analog to SUVs but larger. They have a single lipid bilayer and are used for the encapsulation of large molecules or multiple therapeutic agents.

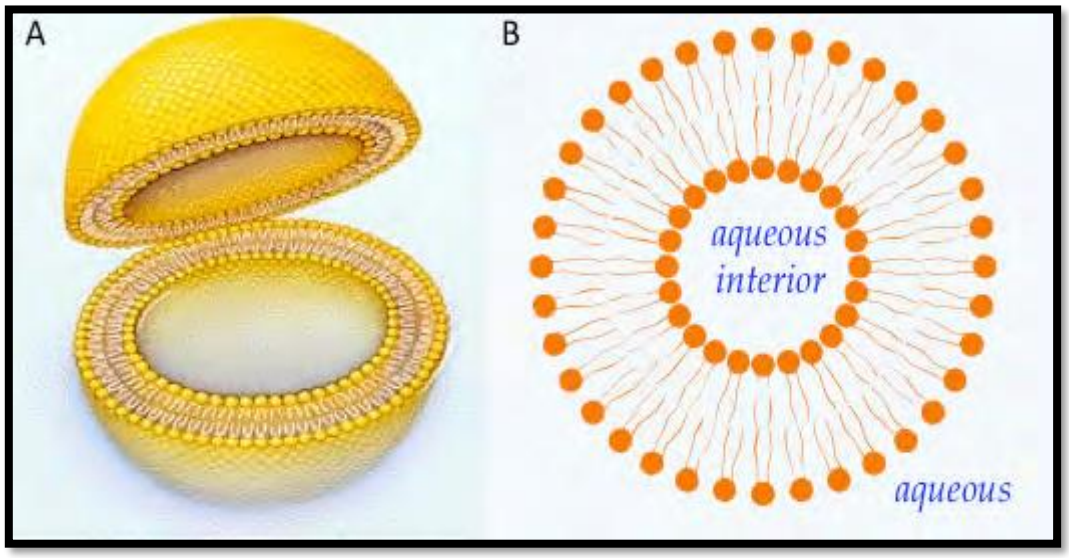


Figure 5: Diagrammatic representation of Large Unilamellar Vesicles (LUVs)[16]

4. **Giant Unilamellar Vesicles (GUVs):** GUVs are very large liposomes, often ranging from 1 to 100 micrometers in diameter. They are typically used in biophysical studies and membrane research due to their size and structural simplicity.

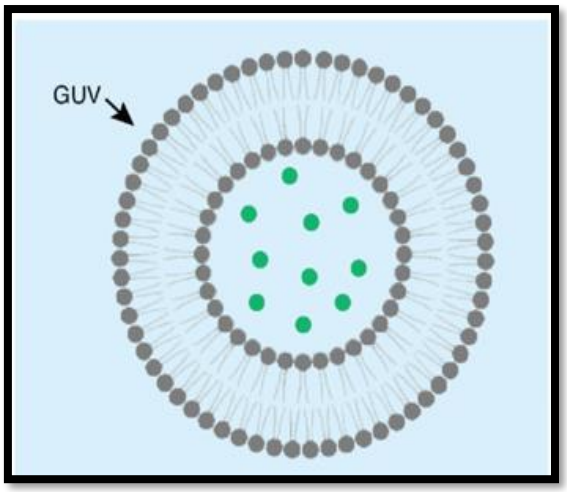


Figure 6: Diagrammatic -representation of Giant Unilamellar Vesicles (GUVs)[17]

5. **Activation-Sensitive Liposomes:** In response to certain stimuli like pH, temperature, enzymatic activity, or light, these liposomes can alter their shape or release their contents. For applications including controlled release and targeted drug administration, stimuli-responsive liposomes have been created.

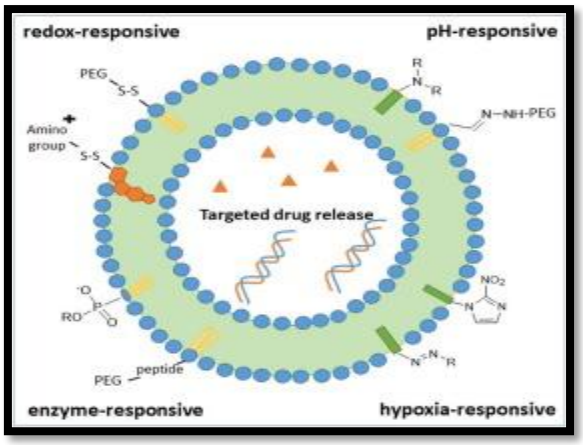


Figure 7: A diagrammatic representation of Stimuli-Responsive Liposomes[18]

6. **PEGylated Liposomes:** PEGylated liposomes have a surface coating of polyethylene glycol (PEG). PEGylation improves liposome stability, prolongs circulation time in the bloodstream, and reduces immune recognition.

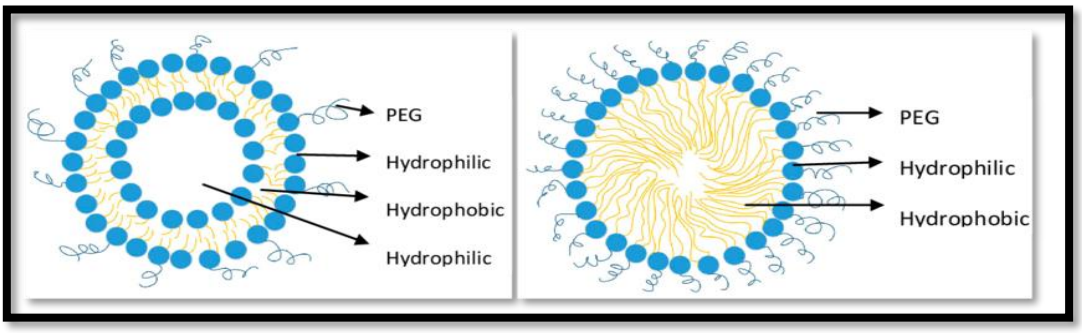


Figure 8: A diagrammatic -representation of PEGylated Liposomes

7. **Cationic Liposomes**: Cationic liposomes have positively charged lipid components, which interact with negatively charged cell membranes. Their ability to effectively encapsulate and transfer nucleic acids into cells makes them useful for gene delivery and nucleic acid transfection.

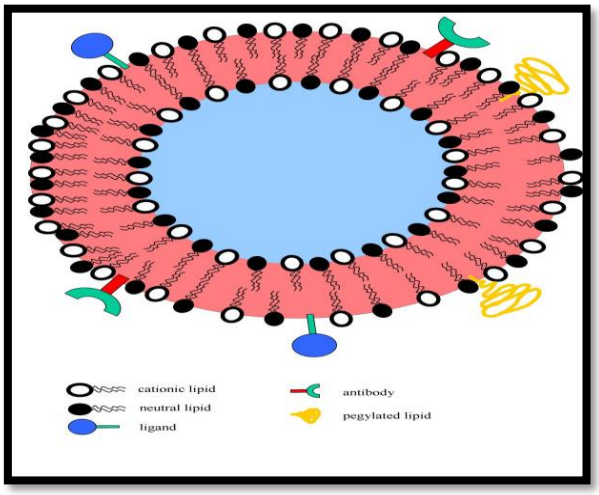


Figure 9: Diagrammatic representation of Cationic Liposomes[18,19]

8. **Hybrid Liposomes:** Hybrid liposomes incorporate additional components such as polymers, peptides, or inorganic nanoparticles into their lipid bilayers. Hybrid liposomes combine the advantages of liposomal drug delivery with the functionalities of other materials for enhanced therapeutic effects.

- **Composition of Liposomes:**[20,21,22]
- **Lipids and phospholipids are employed for the formation of liposomes**

Liposomes are spherical or multilayered spherical vesicles that are created when a lipid bilayer is composed of phospholipids with diacyl chains in aqueous solutions self-assembles. These phospholipids self-organize into an amphiphilic structure by arranging themselves into a lipid bilayer with hydrophilic heads facing outward and hydrophobic tails facing inward. Phospholipids from both naturally occurring and artificially manufactured sources make up liposomes the properties of liposomes, such as stiffness, fluidity, stability, electrical charge, and particle size, are significantly influenced by lipid structure. For example, liposomes made from naturally occurring unsaturated phosphatidylcholine, such that found in eggs or soybeans, have limited stability and high permeability. Liposomes based on saturated phospholipids, such as dipalmitoyl phosphatidylcholine, on the other hand, form hard, nearly impenetrable bilayer structures.

The hydrophilic group found in lipids can have one or more charges—positive, negative, or zwitterionic, meaning that the molecule has both positive and negative charges. Stability is provided by the hydrophilic group's charge-repelling electrostatically. In Figure 10 it illustrates how the saturation, symmetry, and acyl chain length of the hydrophobic lipid group vary.

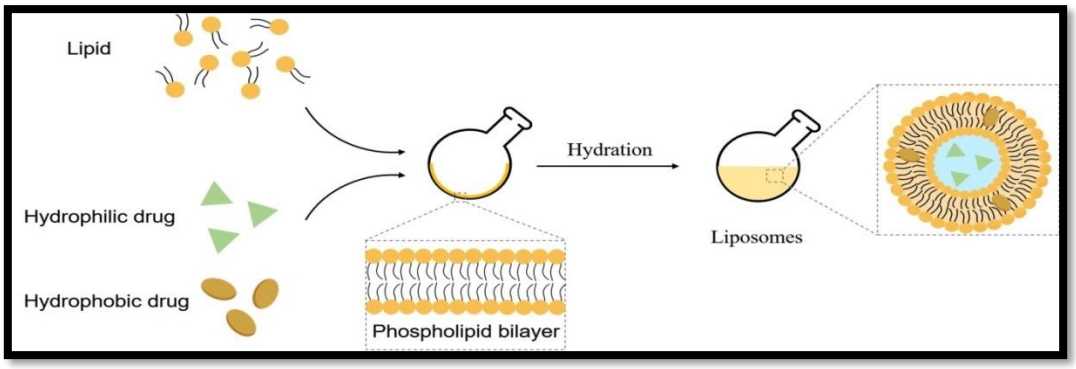


Figure 10: Flowchart representation of liposome formation and encapsulation of drug molecules

- **Organic fats**

Normal cells' lipid bilayer is mostly made up of glycerophospholipids. The structure of these phospholipids revolves around two molecules of fatty acid and a unit of glycerol connected to a phosphate group (PO42-). Furthermore, the phosphate group can form bonds with tiny, necessary chemical molecules such as choline (Figure 11). Phospholipids are found in natural sources like soybean and egg yolk. They are classified as phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI),

phosphatidylserine (PS), phosphatidic acid (PA), and phosphatidylglycerol (PG) depending on whether or not they have polar head groups.

Given their unsaturated hydrocarbon chains, natural phospholipids are less stable than synthetic equivalents when it comes to liposome formation (Figure 11). These naturally occurring phospholipids are composed of a variety of fatty acids, including unsaturated ones like oleic acid, which is found in egg yolk lecithin, and saturated ones like palmitic acid (hexadecanoic acid, H3C-(CH2)14-COOH). Polyunsaturated fatty acids C 20:4 (n-6) and C22:6 (n-3) is prevalent in egg phospholipids. PCs and egg-derived phospholipids are mostly constituted of fatty acids such as oleic acid (C18:1), stearic acid (C18:0), palmitic acid (C16:0), arachidonic acid (C20:4), and linoleic acid (C18:2). Phosphatidylcholine 1-palmitoyl-2-oleoyl comprises around 40% of egg PC.[22]

Stearic acid (C18:0), oleic acid (C18:1), palmitic acid (C16:0), and linolenic acid (C18:3) and linoleic acid (C18:2) makeup approximately 95% of the fatty acid content of phospholipids obtained from soybeans. Interestingly, unsaturated fatty acids, which occupy the α- and β-positions of the glycerol moiety in PC, PE, and PS, account for 50% of the total acids in this phospholipids.[23]

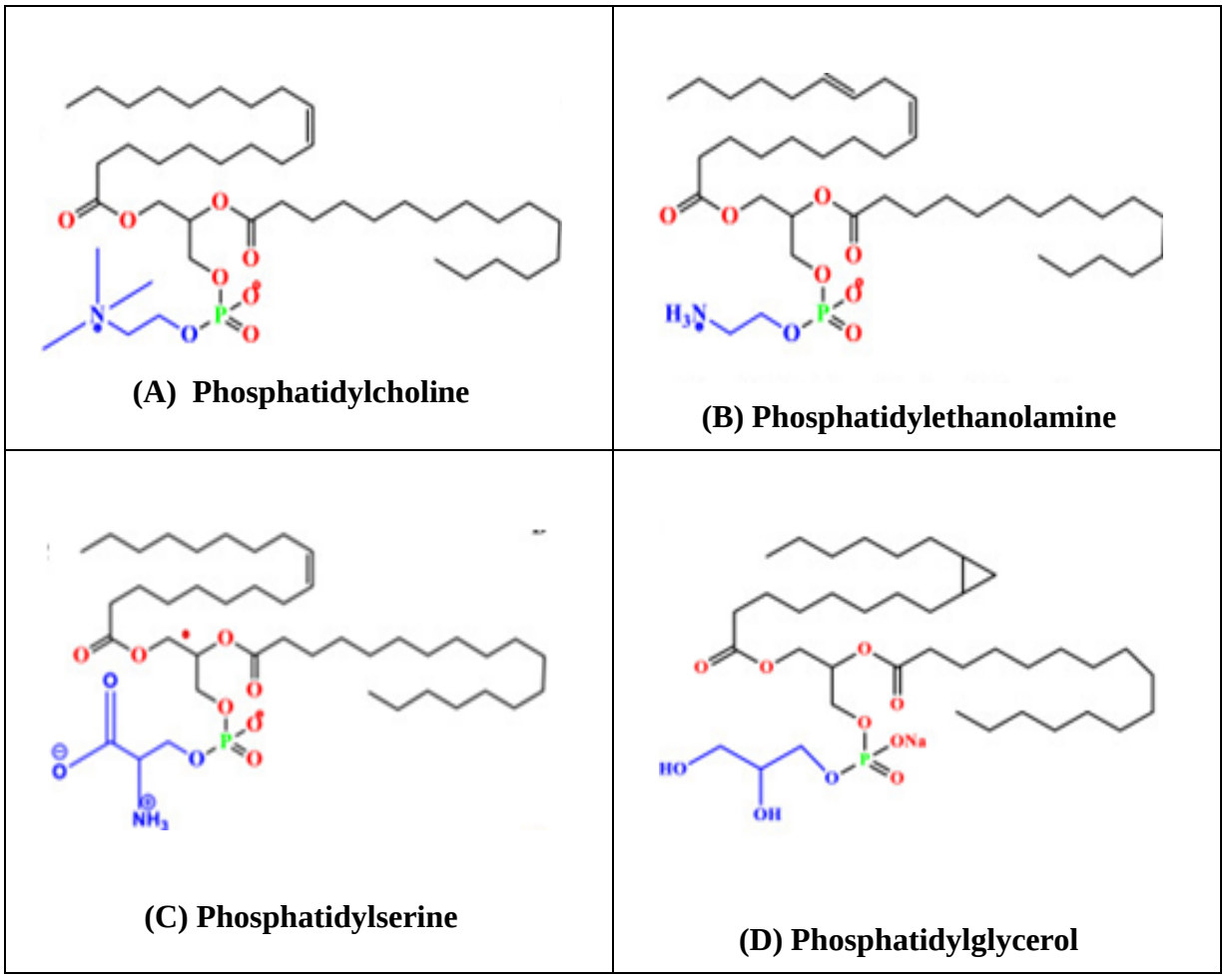
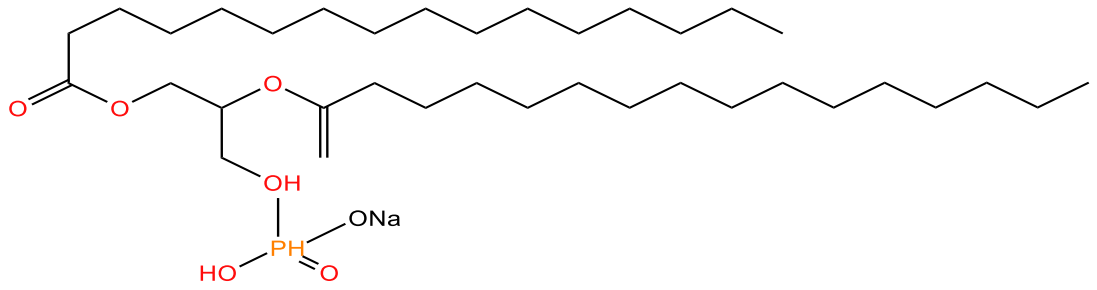


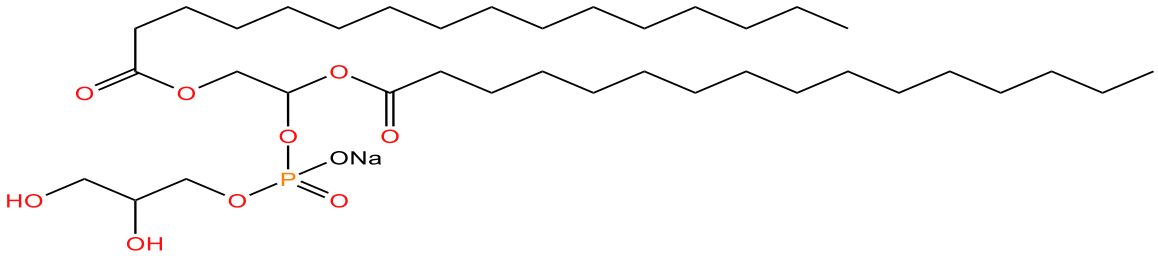
Figure 11: Some examples of Natural phosphatides with their chemical composition: A) Phosphatidylcholine, B) Phosphatidylethanolamine, C) Phosphatidylserine, and D) Phosphatidylglycerol.[24]

- **Synthetic lipids**

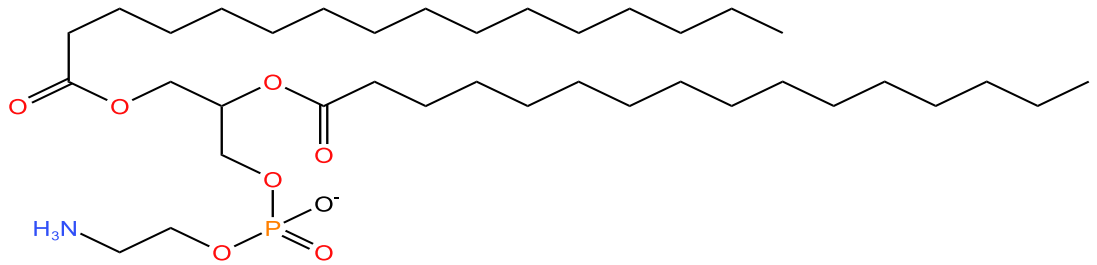
The polar and non-polar portions of natural phospholipids are chemically modified to form synthetic phospholipids. These modifications allow for the synthesis of a wide range of distinct and well-characterized phospholipids. Saturated synthetic phospholipids are mainly derived from Stearic and palmitic fatty acids. As seen in Figure 12, it illustrates various potential synthetic phospholipids commonly employed in liposome formulation. Moreover, unsaturated and mixed fatty acids in either one or both hydrocarbon chains can be used to create synthetic phospholipids.



(A) 1,2-Dipalmitoyl-sn-glycero-3-phosphatidic acid sodium salt (DPPA)



(B) 1,2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt (DPPG)



(C) 1,2-Dipalmitoyl-sn-glycero-3-phosphorylethanolamine (DPPE)

Figure 12: Synthesized phospholipids with their chemical composition based on palmitic acid; A) 1, 2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt, (B) 1, 2-Dipalmitoyl-sn-glycero-3-phosphorylglycerol sodium salt, (C) 1, 2-Dipalmitoyl-sn-glycero-3-phosphorylethanolamine (DPPE).[25,26]

- **Steroid**

Steroids are hydrophobic lipids with four-ring topologies, as seen in Figure 13. The several functional groups that are affixed to those rings are the source of steroids' variety. Since cholesterol is a component of the lipid bilayer of liposomes, it is the primary steroid utilized in liposome synthesis, accounting for less than 30% of total lipids. This helps to enhance the hardness and stability of liposomes.

A comparative analysis of the effects of cholesterol and β-Sitosterol on the characteristics of liposome membranes revealed that both steroids raise absolute zeta potential and decrease the fluidity of the liposome membrane, leading to notable modifications in enthalpy, particle size, and DPPC phase transition temperature (Tm).

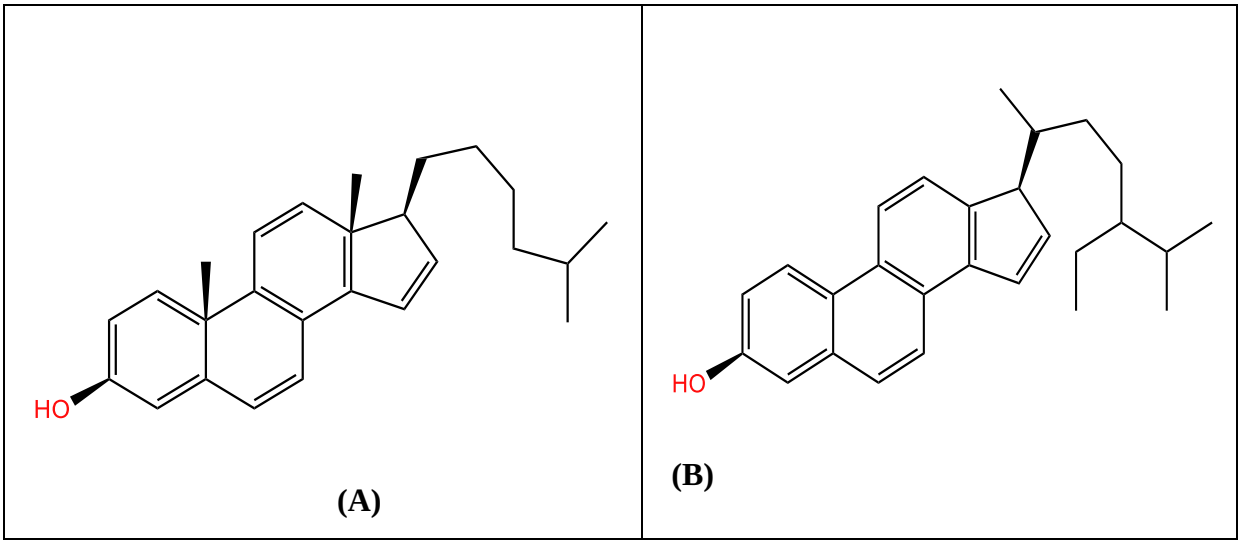


Figure 13: Chemical composition of A) cholesterol, B) β-sitosterol.[27, 28]

- **Surfactants**

While surfactants reduce the surface tension between incompatible phases that do not mix well, they can alter how liposomes encapsulate and release molecules. The surfactants with amphiphiles with a single acyl chain increase the flexibility of nanocarriers by disrupting the lipid bilayer of 'liposomal nanoparticles' (Figure 14). In liposome formulations, Surfactants are commonly used (Span 60, Span 80, and Tween 60, and Tween 80, sodium cholate). Liposomes containing different surfactants are widely utilized to improve the penetration of drugs encapsulated within them through the skin. Transfersomes are a type of surfactant-based Nano vesicle it shows promise in delivering drugs through the skin and is also known as ultra-deformable liposomes. The term "edge activator," synonymous with surfactant, refers to its role in enhancing the deformability of liposomes by modifying their lipid bilayers.

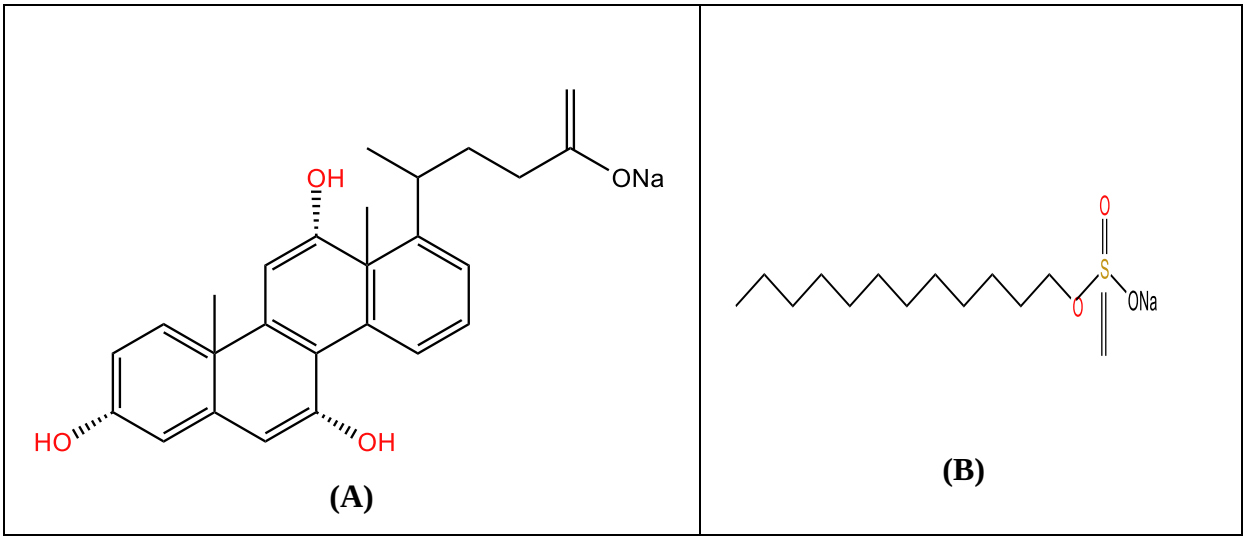


Figure 14: Chemical composition of surfactants A) sodium cholate, B) Sodium dodecyl sulfate (SDS) [20, 28]

- **Methods of preparation**[29, 30]

Various techniques can be employed to produce liposomes. The final liposome features are significantly influenced by the phospholipid type and liposome manufacturing procedure. Liposome's fabrication methods are classified into:

1. **The Bangham technique of thin-film hydration**: -

Using a round-bottom flask, lipids, and the hydrophobic medication are dissolved in the proper organic solvent in this approach. The organic solvent is then slowly evaporated under low pressure to create a thin coating. After that, an aqueous buffer solution above the lipid's transition temperature (Tm) is employed to hydrate this thin layer.

Drugs that are hydrophilic and meant to be loaded into the liposomes' watery core can be included in the hydration solution. The effectiveness of drug encapsulation is significantly influenced by the rate of hydration; slower rates of hydration often translate into better encapsulation efficiencies.

Extrusion through polycarbonate membranes with certain whole diameters or the use of bath or probe sonicators can be used to regulate the size, distribution, and lamellarity of liposomes. When compared to sonication, extrusion yields more stable liposomes with higher encapsulation efficiency.

Small unilamellar vesicles (SUVs) are usually produced by sonication, but it can also cause lipids and/or medications that are encapsulated to break down or hydrolyze. Furthermore, there is a chance that liposome suspensions are contaminated with metals due to probe sonication (Figure 15).

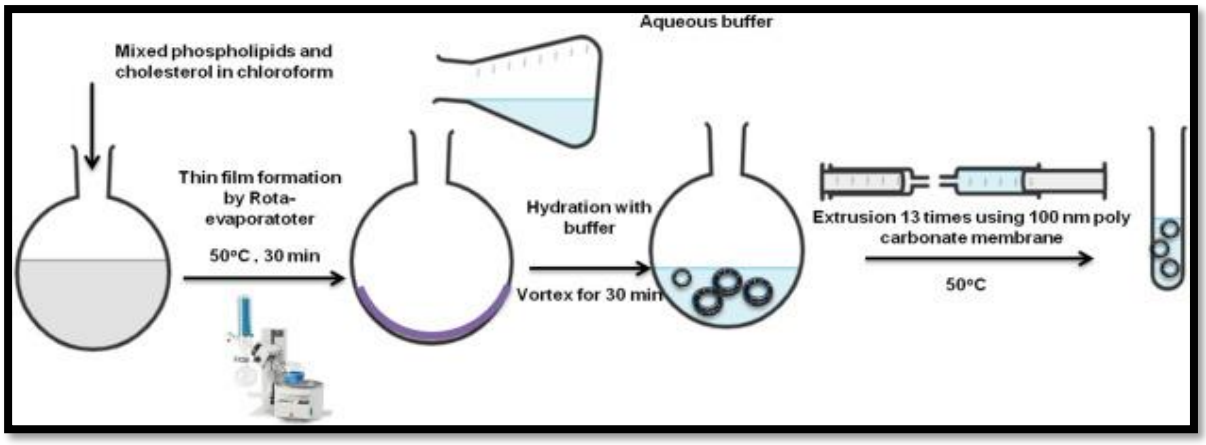


Figure 15: preparation of Liposome by using thin-film hydration extrusion method

2. The Dehydration- Rehydration Method[31,32]

This method involves initially rehydrating SUVs (Small Unilamellar Vesicles) in an empty buffer with an aqueous solution containing the desired material for encapsulation, followed by drying. This process results in a dispersion of solid lipids in finely divided form, often employing freeze-drying as a preferred technique. Subsequently, the vesicles are rehydrated, yielding liposomes typically characterized as oligolamellar vesicles.

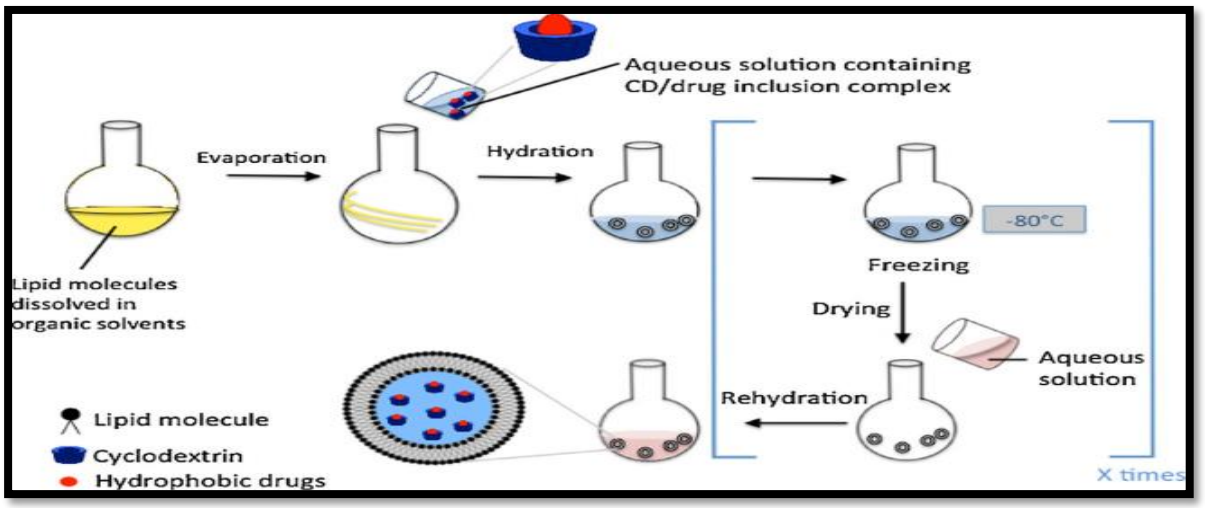


Figure 16: preparation of liposomes by using Dehydration- Rehydration Method

3. Method of reverse-phase evaporation

By creating an emulsion of water and oil, the technique is often utilised in place of thin-film hydration. First, the lipids are dissolved in organic solvents, and then the hydrophilic medication is immediately dissolved in an aqueous buffer.

Lipid vesicles are created when the organic solvent evaporates in the rotary evaporator at a lower pressure, and these vesicles are subsequently distributed throughout the aqueous solution. The preformed vesicles' average size and polydispersity can be reduced via extrusion. Only large molecular weight molecules should be used with this technique since the therapeutic peptides may become denatured by the organic solvents and sonication conditions.

4. Techniques for injecting solvents

The injection techniques were classified based on the kind of organic solvent that was utilized to create the liposomes (Figure 17). As in this instance, the hydrophobic active agents and lipids were quickly introduced into an aqueous phase, and the organic solvent was utilized to dissolve them. Diethyl ether allows direct solvent evaporation to occur during mixing at a temperature higher than the solvent's boiling point.

Ethanol is vacuum-evaporated using a rotary evaporator, dialysis, or filtration since it is needed for the injection of 10 to 20 factorial of an aqueous solution. Most liposomal formulations with greater polydispersity indices (PDI) are produced using this technique. Its constant exposure to high temperatures and chemical solvents may reduce the stability of lipids and drugs.[33]

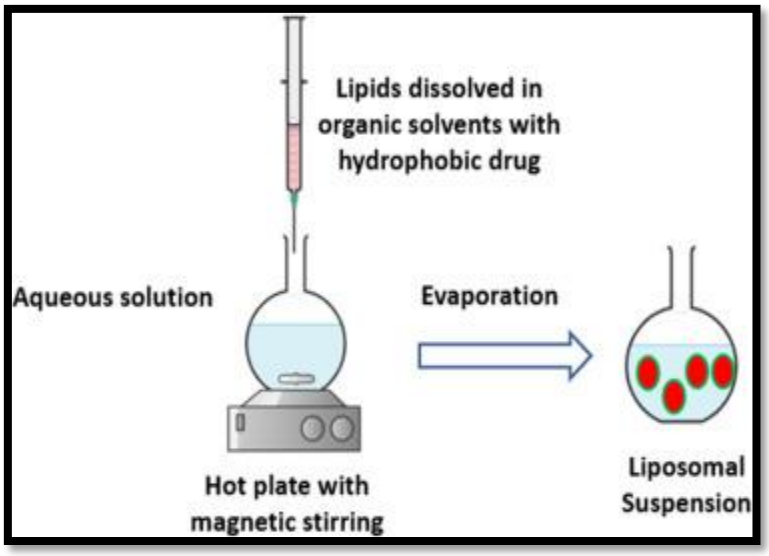


Figure 17: Flow chart representation of injection methods method.

5. Method to eliminate detergents[34]

This approach involves dissolving lipids and a high critical micelle concentration (CMC) in an appropriate organic solvent using a round-bottom flask. A thin lipid coating forms at the bottom of the flask as a result of the solvent's slow evaporation. After that, an aqueous solution containing the required drug molecules is added to the lipid layer, hydrating it and causing mixed micelles to form. The surfactant is then extracted from the mixture using techniques including dialysis, size-exclusion chromatography, dilution, or adsorption onto hydrophobic beads. This stage is essential because it makes the transition from micelles to bigger liposomes easier.

Following surfactant removal, the solution is concentrated to obtain large unilamellar vesicles (LUVs), forming the final liposomal vesicles. However, a notable disadvantage of this method is the potential separation of hydrophilic drugs from the liposomes during the surfactant removal step. This can reduce the encapsulation efficiency of hydrophilic compounds and requires optimization to minimize drug loss.

6. pH jumping method[35, 25]

This approach converts MLVs into SUVs by subjecting the aqueous solution of phosphatidylcholine and phosphatidic acid to almost four-fold due to which there is an increase in pH over a brief period. The proportion of SUVs to LUVs produced is determined by the phosphatidic acid to phosphatidyl choline ratio.

- **Advantages of Liposomes:**

1. **Biocompatibility**: Liposomes are not causing harm and are biocompatible with cells and tissues since they are made up of synthetic or natural phospholipids.
2. **Versatile Drug Delivery**: They can encapsulate medicines that are both hydrophilic and hydrophobic, permitting a wide range of application in drug delivery applications.
3. **Targeted Delivery**: By altering liposomes to target particular tissues or cells, systemic side effects can be reduced and therapeutic efficacy can be increased.
4. **Enhanced Pharmacokinetics**: They enhance a drug's physicochemical profile by prolonging circulation time in the bloodstream, thereby increasing drug bioavailability.
5. **Protection of Drugs**: Liposomes protect encapsulated drugs from degradation and metabolism, improving stability and ensuring controlled release.
6. **Reduced Toxicity**: Liposomal formulations can reduce the toxicity of drugs by targeting delivery to diseased tissues while sparing healthy cells.
7. **Flexibility in Formulation**: Liposomes can be modified in terms of surface charge, composition, and size, and encapsulation efficiency to suit specific therapeutic needs.
8. **Stimuli-Responsive Properties**: Drug delivery and efficacy can be enhanced by stimuli-responsive liposomes, which release medications in response to particular triggers like pH, temperature, or enzyme activity.
9. **Enhanced Patient Compliance**: Liposomal formulations can reduce dosing frequency and improve patient compliance due to their sustained release properties.
10. Liposomes are non-toxic and biocompatible to cells and tissues since they are made of synthetic or natural phospholipids.

- **Disadvantages of Liposomes:[36,37,38]**

1. **Storage Instability**: Liposomes can be sensitive to storage conditions such as temperature and pH, affecting their stability and shelf life.
2. **Complex Manufacturing**: The manufacturing process of liposomes can be complex and costly, particularly for large-scale production.
3. **Batch-to-Batch Variability**: There may be variability in liposome characteristics between batches, impacting consistency in drug delivery and efficacy.
4. **Limited Loading Capacity**: Certain medications, particularly those with high molecular weights or poor solubility, may not be fully encapsulated by liposomes.
5. **Recognition by Immune System**: Non-PEGylated liposomes might be recognized by the immune system, prompting the body to eliminate it right away, and reducing efficacy.

6. **Risk of Leakage**: Liposomes can undergo leakage of encapsulated drugs during storage or in vivo, affecting drug release kinetics and therapeutic efficacy.
7. **Difficulty in Scale-up**: Scaling up liposome production from laboratory to commercial scale can pose challenges in maintaining product quality and consistency
8. **Limited Penetration**: Despite advances, liposomes may face challenges in penetrating certain biological barriers or tumour tissues efficiently.
9. **Ethical Concerns**: There may be ethical concerns related to the use of liposomal formulations, especially regarding their long-term safety and environmental impact.

- **APPLICATIONS OF LIPOSOMES**[39,40,41]

1. Drug Delivery

Liposomes' distinct shape and characteristics have made them well-known for their use in medication delivery. They consist of one or more lipid bilayers that can store both hydrophilic and hydrophobic medicines, around an aqueous core. Because of their adaptability, liposomes help keep medications from degrading and increase how long they stay in the body's circulation, and target specific tissues or cells. Liposomal formulations have been successfully employed in the delivery of chemotherapy agents, antibiotics, antifungals, and other therapeutic compounds. They improve therapeutic efficacy, reduce systemic toxicity, and enhance patient compliance by enabling the controlled release of drugs at the desired site of action.

2. Vaccine Delivery

In vaccine development, liposomes serve as effective carriers for antigens and adjuvants. They enhance the immunogenicity of vaccines by protecting antigens from enzymatic degradation, facilitating their uptake by antigen-presenting cells (APCs), and promoting antigen presentation to the immune system. Liposomal vaccinations can elicit strong immunological responses, encompassing humoral and cellular immunity, leading to improved vaccine efficacy. They are utilized in the development of vaccines against infectious diseases, cancer immunotherapy, and other therapeutic applications.

3. Cosmetics and Personal Care

Liposomes are used in the cosmetics industry to deliver active substances including antioxidants, vitamins, and moisturizers. Liposomal formulations increase these substances' bioavailability and stability, facilitate their penetration into the skin's layers, and encourage long-term sustained release. This enables targeted delivery of beneficial ingredients to specific skin cells, resulting in enhanced skin hydration, anti-aging effects, and overall skin health improvement. Liposomes also contribute to the development of sunscreens, hair care products, and other cosmetic formulations aimed at improving product efficacy and consumer satisfaction.

4. Diagnostics and Imaging[43, 42]

Liposomes play a crucial role in diagnostic imaging applications by serving as carriers for contrast agents and imaging probes. They can be loaded with fluorescent dyes,

radionuclides, magnetic nanoparticles, or other imaging agents to facilitate precise imaging of tissues and organs. Diagnostic imaging methods like computed tomography (CT), optical imaging, and magnetic resonance imaging (MRI) are made more sensitive and specific by liposomal formulations. They enable early detection of diseases, monitoring of therapeutic responses, and visualization of anatomical structures with high resolution and accuracy.

5. Gene Therapy

In gene therapy, liposomes are utilized as non-viral vectors for delivering therapeutic nucleic acids (DNA or RNA) into target cells. Liposomal formulations protect genetic material from enzymatic degradation and immune recognition, facilitate cellular uptake via endocytosis, and promote intracellular delivery of therapeutic genes. They can be engineered to achieve tissue-specific targeting and controlled release of genetic payloads, thereby enhancing the safety and efficacy of gene-based therapies. Liposomal gene delivery systems are investigated for the treatment of genetic disorders, cancer, and various acquired diseases where precise regulation of gene expression is essential.

6. Nutraceutical and Functional Foods

Liposomes are explored in the field of nutraceutical and functional foods for the delivery of bioactive compounds such as vitamins, antioxidants, and phytochemicals. Liposomal formulations improve the stability, solubility, and bioavailability of these compounds in the gastrointestinal tract, enhancing their absorption and efficacy in the body. They protect sensitive nutrients from degradation due to pH fluctuations and enzymatic activity in the digestive system, thereby maximizing their health benefits. Liposomal nutraceutical are utilized in dietary supplements, fortified foods, and therapeutic formulations aimed at promoting overall health, immune function, and disease prevention.

7. Agricultural and Environmental Applications

In agriculture, liposomes are investigated as carriers for delivering agrochemicals such as pesticides, herbicides, and fertilizers to plants. Liposomal formulations improve the efficacy and targeted delivery of these compounds, reducing environmental contamination and minimizing off-target effects on non-target organisms. They enhance the stability and bioavailability of agricultural inputs in harsh environmental conditions, promoting sustainable farming practices and increasing crop yields.

Liposomes also find applications in environmental remediation by delivering pollutants-degrading enzymes or chemicals to contaminated sites, facilitating efficient degradation and clean-up of environmental pollutants.

- **Marketed formulation of liposome:,**[44,45,46]

- **Marketed formulation of liposomes:** Because liposomes are spherical vesicles with a phospholipids bilayer and may encapsulate, they are often employed as drug delivery vehicles since they are both hydrophilic and hydrophobic medications. These are a few commercially available liposome formulations as shown in table 1.

Table 1: Some Examples of Marketed formulations of liposomes[47, 48, 49]

S. No.	Drugs Name	Indicators	Formulations
1.	**Doxil (Doxorubicin)**	Cancer treatment, the indication includes conditions like multiple myeloma of the breast, cancer of the ovaries, and Kaposi's sarcoma.	To escape the immune system and extend the period that doxorubicin circulates in the bloodstream, it is encapsulated in stealth liposomes that have been PEGylated (coated with polyethylene glycol).
2.	**Ambisome (Amphotericin B)**	Leishmaniasis is one indication of fungus infections.	Compared to other formulations, amphotericin B's toxicity is decreased by being encapsulated in unilamellar liposomes.
3.	**DepoCyt (Cytarabine)**	Lymphomatous meningitis is the indication.	Extended-duration Cytarabine's liposomal composition enables sustained medication delivery straight into the cerebrospinal fluid.
4.	**Marqibo (Vincristine)**	Acute lymphoblastic leukemia (ALL) is the indication.	Sphingomyelin/cholesterol liposomes encapsulating vincristine sulfate, enabling increased dosage and decreased toxicity.
5.	**Vyxeos (Daunorubicin and Cytarabine)**	Acute myeloid leukemia (AML) is the indication.	Combination of daunorubicin and cytarabine contained in liposomes ensures a steady molar ratio and beneficial outcome.

➢ SUMMARY AND CONCLUSION

- **Summary:**

Liposomes have garnered significant attention as a versatile drug delivery system owing to their unique structural composition and biocompatibility. This comprehensive review has delved into various methods utilized for the preparation of liposomes, each influencing their size, stability, and drug encapsulation efficiency. Key methods discussed include reverse-phase evaporation, thin-film hydration, and microfluidic techniques, highlighting their respective advantages and applications in pharmaceutical sciences. Moreover, the review has underscored the importance of surface modification strategies in enhancing the therapeutic efficacy of liposomes by improving targeting capabilities and prolonging circulation times in vivo. Overall, liposomes represent a promising platform for delivering

both hydrophilic and hydrophobic drugs, with continuous advancements in preparation techniques paving the way for more effective and targeted drug delivery systems. Theoretically, combination therapies and personalized medicine could benefit from the co-encapsulation of several medications or therapeutic agents to produce beneficial side effects. Additionally, the therapeutic potential of liposomal technologies is being expanded by developments like stimuli-responsive liposomes, which release pharmaceuticals in response to environmental cues like pH, temperature, and enzymes. But there are still a lot of obstacles to overcome, including batch-to-batch variability, stability in storage, and scalability for large-scale production. It will take multidisciplinary work in the fields of chemistry, materials science, pharmacology, and engineering to solve these problems. In summary, even though liposomes have demonstrated a great deal of promise for drug delivery systems, further investigation is required to perfect their design, enhance production methods, and confirm their safety and effectiveness in clinical settings. Liposomal drug delivery systems have the potential to significantly improve therapeutic results for a range of medical illnesses in the future with further research and cooperation.

- **Conclusion:**

In conclusion, liposomes have demonstrated immense potential as a drug delivery vehicle, offering a wide array of benefits such as controlled release kinetics, biocompatibility and the ability to encapsulate diverse therapeutic agents. The methods discussed in this review ranging from conventional to innovative approaches illustrate the on-going efforts to optimize liposomal formulations for clinical applications. Future research directions may focus on further refining preparation techniques to achieve precise control over liposome characteristics and exploring novel applications such as personalized medicine and combination therapies. By harnessing the capabilities of liposomes and continually advancing their preparation methods, researchers can unlock new possibilities in drug delivery, ultimately improving patient outcomes and quality of life.

Due to their capacity to encapsulate both hydrophobic and hydrophilic pharmaceuticals, liposomes are versatile drug delivery technologies that have drawn a lot of attention. This enhances medication solubility, bioavailability, and targeting. They are made up of lipid bilayers that resemble cell membranes and enclose aqueous compartments. Lipid detergent removal, film hydration, and reverse-phase evaporation are three liposome preparation techniques that have different benefits in terms of encapsulation effectiveness, size control, and scalability.

As drug carriers, liposomes theoretically have several benefits. They can alter pharmacokinetics to improve therapeutic efficacy, prevent drug degradation, and lower toxicity by focusing on delivery. Furthermore, they can be used for a wide range of therapeutic applications, such as the delivery of vaccines or the treatment of cancer, its due to their biocompatibility and capacity to carry a variety of payloads. Despite these benefits, there are still difficulties.

- **REFERENCES:**

1. Verma, R. K., Garg, S., & Current, R. (2001). Drug delivery: Issues and advancements. *Aaps Pharmscitech, 3*(2), E1.
2. Torchilin, V. P. (Ed.). (2010). *Targeted Delivery of Imaging Agents and Medications*. CRC Press.
3. Uhrich, K. E., Cannizzaro, S. M., & Langer, R. S. (1999). Shaping the future of biomaterials. *Biomaterials, 20*(23-24), 2363-2378.
4. Ratnaparkhi, M. P., & Nandave, M. (2016). Novel drug delivery systems: A review. *World Journal of Pharmacy and Pharmaceutical Sciences, 5*(9), 954-968.
5. Jindal, A. B., & Kumar, S. (2013). Novel drug delivery systems and their economics. *International Journal of Research in Pharmacy and Chemistry, 3*(3), 557-566.
6. Mura, S., Nicolas, J., & Couvreur, P. (2013). Stimuli-responsive nanocarriers for drug delivery. *Nature Materials, 12*(11), 991-1003.
7. Soppimath, K. S., Aminabhavi, T. M., & Kulkarni, A. R. (2001). Biodegradable polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release, 70*(1-2), 1-20.
8. peppas, N. A., & Wood, K. M. (2012). Polymeric materials for oral insulin delivery. *Advanced Drug Delivery Reviews, 64*(6), 557-570.
9. Nayak kumar Amit *et al,* (2022). Nanovascular Systems in Drug Delivery. Journal of System of Nanovascular Drug Delivery, (Academic press). PP. 1-15.
10. Jacob, S.; Nair, A.B.; Shah, J.; Gupta, S.; Boddu, S.H.; Sreeharsha, N. 2022 Lipid Nanoparticles as a Promising Drug Delivery Carrier for Topical Ocular Therapy—An Overview on Recent Advances. *Pharmaceutics*, *14*, 533.[CrossRef]
11. Kim, T.; Lee, J.; Jo, Y.J.; Choi, M.J. 2021 Application of Liposome Encapsulating Lactobacillus curvatus Extract in Cosmetic Emulsion Lotion. *Materials*, *14*, 7571. [CrossRef]
12. Lee, J.; Yang, J.H.; Kim, K.S.; Park, G.D. 2021 Effect of Liposome Storage of Cyanocobalamin on Its Degradation by Ascorbic Acid. *Food Suppl. Biomater. Health*, *1*, e7.[CrossRef]
13. Marsanasco, M.; Del Valle Alonso, S. 2021 Why produce food-bioactive compounds to generate functional grade foods? In *Functional Foods-Phytochemicals and Health Promoting Potential*; Muhammad, S.A., Muhammad, H.A., Eds.; IntechOpen: London, UK. [CrossRef]

14. Van Hoogevest, P.; Wendel, A. 2014. The use of natural and synthetic phospholipids as pharmaceutical excipients. *Eur. J. Lipid. Sci. Technol.*, *116*, 1088–1107. [CrossRef]

15. Nakhaei, P.; Margiana, R.; Bokov, D.O.; Abdelbasset, W.K.; Kouhbanani, M.A.J.; Varma, R.S. 2021 Liposomes: Structure, biomedical applications, and stability parameters with emphasis on cholesterol. *Front. Bioeng. Biotechnol.*, *9*, 748. [CrossRef]

16. Karunaratne, D.N.; Pamunuwa, G.K.; Nicholas, I.H.; Ariyarathna, I.R. 2019 Science of Spices and Culinary Herbs-Latest Laboratory. In *Pre-Clinical, and Clinical Studies*; Atta-ur-Rahman, Choudhary, M.I., Yousuf, S., Eds.; Bentham Science Publishers: Sharjah, United Arab Emirates; Volume 1, pp. 104–147. [CrossRef].

17. Subramanian, P. 2021 Lipid-based nanocarrier system for the effective delivery of nutraceuticals. *Molecules*, *26*, 5510. [CrossRef]

18. Nguyen, T.X.; Huang, L.; Liu, L.; Abdalla, A.M.E.; Gauthier, M.; Yang, G. 2014 Chitosan-coated nano-liposomes for the oral delivery of berberine hydrochloride. *J. Mater. Chem. B*, *2*, 7149–7159. [CrossRef]

19. Pentak, D.; Ploch-Jankowska, A.; Zięba, A.; Kozik, V. 2022 The Advances and Challenges of Liposome-Assisted Drug Release in the Presence of Serum Albumin Molecules: The Influence of Surrounding pH. *Materials*, *15*, 1586.[CrossRef]

20. Mirzavi, F.; Barati, M.; Soleimani, A.; Vakili-Ghartavol, R.; Jaafari, M.R.; Soukhtanloo, M. 2021 A review on liposome-based therapeutic approaches against malignant melanoma. *Int. J. Pharm.*, *599*, 120413. [CrossRef]

21. Wang, G.; Li, R.; Parseh, B.; Du, G. 2021 Prospects and challenges of anticancer agents' delivery via chitosan-based drug carriers to combat breast cancer: A review. *Carbohydr. Polym*, *268*, 118192.[CrossRef]

22. Watson, D.S.; Endsley, A.N.; Huang, L. 2012 Design considerations for liposomal vaccines: Influence of formulation parameters on antibody and cell-mediated immune responses to liposome associated antigens. *Vaccine*, *30*, 2256–2272.[CrossRef]

23. Man, F.; Gawne, P.J.; de Rosales, R.T.M. 2019 Nuclear imaging of liposomal drug delivery systems: A critical review of radiolabelling methods and applications in nanomedicine. *Adv. Drug Delivery Rev.*, *143*, 134–160.[CrossRef]

24. Taha, E.I.; El-Anazi, M.H.; El-Bagory, I.M.; Bayomi, M.A. 2014 Design of liposomal colloidal systems for ocular delivery of ciprofloxacin. *Saudi Pharm. J.*, *22*, 231–239. [CrossRef]

25. Han, Y.; Gao, Z.; Chen, L.; Kang, L.; Huang, W.; Jin, M.; Wang, Q.; Bae, Y.H. 2014 Multifunctional oral delivery systems for enhanced bioavailability of therapeutic peptides/proteins. *Acta Pharm. Sin. B*, *9*, 902–922. [CrossRef]

26. Mirtaleb, M.S.; Shahraky, M.K.; Ekrami, E.; Mirtaleb, A. 2021 Advances in biological nano-phospholipid vesicles for transdermal delivery: A review on applications. *J. Drug Delivery Sci. Technol.*, *61*, 102331. [CrossRef]

27. Mehta, P.P.; Ghoshal, D.; Pawar, A.P.; Kadam, S.S.; Dhapte-Pawar, V.S. 2020 Recent advances in inhalable liposomes for treatment of pulmonary diseases: Concept to clinical stance. *J. Drug Delivery Sci. Technol.*, *56*, 101509.[CrossRef]

28. Ahmad, A.; Ahsan, H. 2020 Lipid-based formulations in cosmeceuticals and biopharmaceuticals. *Biomed. Dermatol.*, *4*, 1–10.[CrossRef]

29. Padilla, C.E.; Villanueva, F.J.E. 2018 Encapsulation of food active ingredients in liposomes. *J. Nutr. Health. Food. Eng. 8*, 238–239. [CrossRef]

30. Liu, W.; Hou, Y.; Jin, Y.; Wang, Y.; Xu, X.; Han, J. 2020 Research progress on liposomes: Application in food, digestion behavior and absorption mechanism. *Trends Food Sci. Technol.*, *104*, 177–189.[CrossRef]

31. Torchilin, V. P. (2005). Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery*, *4*(2), 145-160.

32. Arpita, S.; Kumar, S.V. Liposomes–A Review. *Int. J. Indig. Herbs Drugs* 2020, *5*, 1–6. [CrossRef]

33. Paliwal, R.; Paliwal, S.R.; Kenwat, R.; Kurmi, B.D.; Sahu, M.K. 2020 Solid lipid nanoparticles: A review on recent perspectives and patents. *Expert. Opin. Ther. Pat.*, *30*, 179–194. [CrossRef]

34. Ajeeshkumar, K.K.; Aneesh, P.A.; Raju, N.; Suseela, M.; Ravishankar, C.N.; Benjakul, S. 2021 Advancements in liposome technology: Preparation techniques and applications in food, functional foods, and bioactive delivery: A review. *Compr. Rev. Food. Sci. Food Saf.*, *20*, 1280–1306. [CrossRef]

35. Lazuardi, M.; Suharjomo, S.; Chien, C.H.; He, J.L.; Sugihartuti, R.; Maslachah, L. 2022 Encapsulation of progesterone-like compounds in 10% liposome increases their concentration in rats administered an injectable dosage form of these compounds. *Kafkas Univ. Veter-Fak. Derg.*, *28*, 27–34. [CrossRef]

36. De Freitas, C.F.; Calori, I.R.; Tessaro, A.L.; Caetano, W.; Hioka, N. 2019 Rapid formation of small unilamellar vesicles (suv) through low-frequency sonication: An innovative approach. *Colloid. Surf. B*, *181*, 837–844. [CrossRef]

37. Subramani, T.; Ganapathyswamy, H. 2020 An overview of liposomal nano-encapsulation techniques and its applications in food and nutraceutical. *J. Food. Sci. Technol.*, *57*, 3545–3555.[CrossRef]

38. Sheoran, R.; Khokra, S.L.; Chawla, V.; Dureja, H. 2019 Recent patents, formulation techniques, classification and characterization of liposomes. *Recent Pat. Nanotechnol.*, *13*, 17–27. [CrossRef]

39. Trucillo, P.; Reverchon, E. 2021 Production of PEG-coated liposomes using a continuous supercritical assisted process. *J. Supercrit. Fluids*, *167*, 105048. [CrossRef]

40. Rashidinejad, A.; Jafari, S.M. 2020 Nanoencapsulation of bioactive food ingredients. In *Handbook of Food Nanotechnology*; Jafari, S.M., Ed.; Academic Press: Cambridge, MA, USA; pp. 279–344. [CrossRef]

41. Saudagar, R.B.; Saokar, S. Anti-inflammatory natural compounds from herbal and marine origin. *J. Drug Deliv. Ther.* 2019, *9*, 669–672. Available online: https://jddtonline.info/index.php/jddt/article/view/2906 (accessed on 15 April 2022).

42. Kelly C. Jefferies C. Cryan S.-A.J. 2011Targeted Liposomal Drug Delivery to Monocytes and Macrophages. *J. Drug Deliv.*

43. Caracciolo G., Pozzi D.,Caminiti R.,Amenitsch H. 2005 Lipid mixing upon deoxyribonucleic acid-induced liposomes fusion investigated by synchrotron small-angle x-ray scattering. *Appl. Phys. Lett*; 87: 133901

44. Storm G., Belliot S.O.,Daemen T., Lasic D.D. 1995 Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. *Adv. Drug Deliv. Rev.*; 17: 31-48

45. Bozzuto G. ,Molinari A. 2015 Liposomes as nanomedical devices. *Int. J. Nanomed.*; 10: 975

46. Düzgüneş N., Nir S. 1999 Mechanisms and kinetics of liposome–cell interactions. *Adv. Drug Deliv. Rev.*; 40: 3-18

47. Lee K.D.,Nir S.,Papahadjopoulos D. 1993 Quantitative analysis of liposome-cell interactions in vitro: rate constants of binding and endocytosis with suspension and adherent J774 cells and human monocytes, *Biochemistry.*; 32: 889-899.

48. Kono K.,Nakai R.,Morimoto K.,Takagishi T. 1999 Temperature-dependent interaction of thermo-sensitive polymer-modified liposomes with CV1 cells. *FEBS Lett.*; 456: 306-310.

49. Wu X., Dai X, *et al*, 2021Investigation on drug entrapment location in liposomes and transfersomes based on molecular dynamics simulation. *J. Mol. Model.*; 27: 111.