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SIGNIFICANCE OF VIROSOMES ON TARGETED DRUG DELIVERY

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INTRODUCTION

Targeted Drug Delivery, sometimes called smart drug delivery, is a method of delivering medication to a patient in a manner that increases the concentration of the medication in some parts of the body relative to others. This means of delivery is largely founded on nanomedicine, which plans to employ nanoparticle-mediated drug delivery in order to combat the downfalls of conventional drug delivery. These nanoparticles would be loaded with drugs and targeted to specific parts of the body where there is solely diseased tissue, thereby avoiding interaction with healthy tissue. The goal of a targeted drug delivery system is to prolong, localize, target and have a protected drug interaction with the diseased tissue. The conventional drug delivery system is the absorption of the drug across a biological membrane, whereas the targeted release system releases the drug in a dosage form. The advantages to the targeted release system are the reduction in the frequency of the dosagestaken by the patient, having a more uniform effect of the drug, reduction of drug side-effects, and reduced fluctuation in circulating drug levels. The disadvantage of the system is high cost,which makes productivity more difficult, and the reduced ability to adjust the dosages.[1]

In traditional drug delivery systems such as oral ingestion or intravascular injection, the medication is distributed throughout the body through the systemic blood circulation. For most therapeutic agents, only a small portion of the medication reaches the organ to be affected, such as in chemotherapy where roughly 99% of the drugs administered do not reach the tumore site. Targeted drug delivery seeks to concentrate the medication in the tissues of interest while reducing the relative concentration of the medication in the remaining tissues. For example, by avoiding the host's defence mechanisms and inhibiting non-specific distribution in the liver and spleen, a system can reach the intended site of action in higher concentrations. Targeted delivery is believed to improve efficacy while reducing side-

effects.[3]

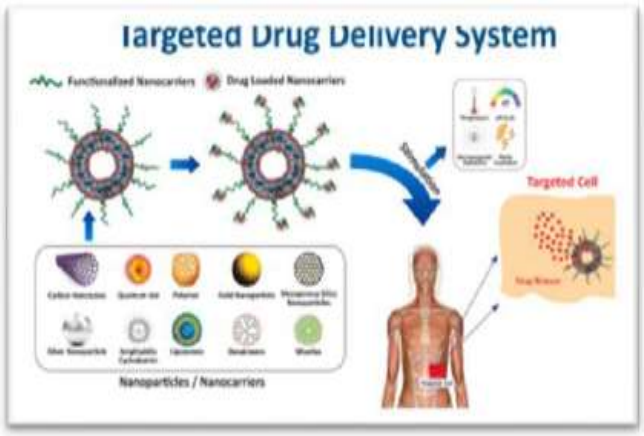


Fig. no. 1: Tergated drug delivery system.

When implementing a targeted release system, the following design criteria for the system must be taken into account: the drug properties, side-effects of the drugs, the route taken forthe delivery of the drug, the targeted site, and the disease.[5]

Increasing developments to novel treatments requires a controlled microenvironment that is accomplished only through the implementation of therapeutic agents whose side-effects can be avoided with targeted drug delivery. Advances in the field of targeted drug delivery to cardiac tissue will be an integral component to regenerate cardiac tissue.

There are two kindsof targeted drug delivery

Active Targeted Drug Delivery, such as some antibody medications, and Passive Targeted Drug Delivery, such as the enhanced permeability and retention effect (EPR-effect).[4] Drug delivery to the body can be broadly classified into two categories: locaand systemic. Local drug delivery is limited to external sites of the body, concentrations to the bloodstream, which allows them to reach the intended site as well as other areas of the body. However, this exposure to other body parts can cause side-effects, and maintaining a therapeutic concentration requires a large dose of the drug. Additionally, treating chronic diseases can pose another challenge, as it requires a steady drug concentration over a longer period of time. Multiple doses can cause the drug concentration to fall below therapeutic levels. To overcome these issues, nanoparticles can be used to deliver drugs more effectively. problems associated with the conventional systemic delivery of the drugs there is a need for the development of a targeted drug delivery system - a systemthat can deliver the drug selectively to the diseased site in a specified steady concentration for the prescribed time.[3]

Drug targeting has been classified into three types

- *First order targeting* - This describes delivery to a desecrate organ or tissue.
- *Second order targeting* - This represents targeting a specific cell type (s) with in tissue or organ.
- *Third order targeting* - Implies delivery to specific intracellular compartments in target cells e.g., Lysosomes.[2]

Basically, there are three approaches for drug targeting

1) The *first approach* involves the use of biologically active agents that are both potent and selective to a particular site in the body (Magic bullet approach).
2) The *second approach* involves the preparation of a pharmacologically inert form of active drugs that when it reaches the active sites becomes activated by a chemical or every enzymatic reaction (Prodrugs approach).

The *third approach* utilizes a biologically inert macromolecular carrier system that directs a drug to a specific site in the body where it is accumulated and affects its response (carrier approach).[2]

Targeting with drug carrier system is divided into **three** types; **1) Passive 2) Active 3) Physical targeting.** ***Passive targeting*** **–** Relies on the normal distribution pattern of the drug carrier system. Ex- particles of 5 µm or smaller are readily removed from the blood by macrophages of RES when administered systemically, this natural defense mechanism of RES thus provides an opportunity to target drug encapsulated in or conjugated to an appropriate carrier system to macrophages. Mechanical filtration of large carriers by capillary blockage can also be exploited to target drugs at the lungs by the venous supply and at other organs through appropriate arterial supply.[7] Passive targeting is achieved by incorporating the therapeutic agent into a macromolecule or nanoparticle that passively reaches the target organ. In passive targeting, the drug's success is directly related to circulation time. This is achieved by cloaking the nanoparticle with some sort of coating. Several substances can achieve this, with one of them being polyethylene glycol (PEG). By adding PEG to the surface of the nanoparticle, it is rendered hydrophilic, thus allowing water molecules to bind to the oxygen molecules on PEG via hydrogen bonding. The result of this bond is a film of hydration around the nanoparticle which makes the substance antiphagocytic. The particles obtain this property due to the hydrophobic interactions that are natural to the reticuloendothelial system (RES), thus the drug-loaded nanoparticle is able to stay in

circulation for a longer period of time. To work in conjunction with this mechanismof passive targeting, nanoparticles that are between 10 and 100 nanometres in size have been found to circulate systemically for longer periods of time.[8]

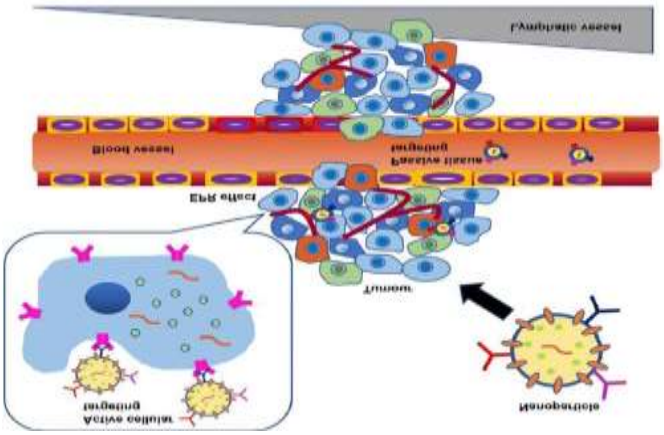


Fig. no. 2: Passive tergating.

Active targeting – Employs a deliberately modified drug carrier molecule capable of recognizing and interacting with specific cell tissue or organ in the body e.g., antigen specific antibody.

Active targeting of drug-loaded nanoparticles enhances the effects of passive targeting to make the nanoparticle more specific to a target site. There are several ways that active targeting can be accomplished. One way to actively target solely diseased tissue in the body is to know the nature of a receptor on the cell for which the drug will be targeted to. Researchers can then utilize cell-specific ligands that will allow the nanoparticle to bind specifically to the cell that has the complementary receptor. This form of active targeting was found to be successful when utilizing transferrin as the cell-specific ligand. The transferrinwas conjugated to the nanoparticle to target tumor cells that possess transferrin-receptor mediated endocytosis mechanisms on their membrane. This means of targeting was found to increase uptake, as opposed to non-conjugated nanoparticles. Another cell-specific ligand is the RGD motif which binds to the integrin αvβ3. This integrin is upregulated in tumor and activated endothelial cells. Conjugation of RGD to chemotherapeutic-loaded nanoparticles has been shown to increase cancer cell uptake in vitro and therapeutic efficacy in vivo.[9] Active targeting can also be achieved by utilizing magneto liposomes, which usually serves as a contrast agent in magnetic resonance imaging. Thus, by grafting these liposomes with a desired drug to deliver to a region of the body, magnetic positioning could aid with this

process.[10]

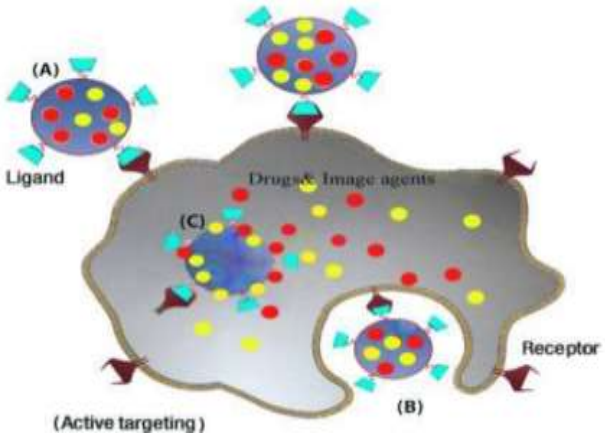


Fig. no. 3: Active targeting.

Furthermore, a nanoparticle could possess the capability to be activated by a trigger that is specific to the target site, such as utilizing materials that are pH responsive. Most of the body has a consistent, neutral pH. However, some areas of the body are naturally more acidic than others, and, thus, nanoparticles can take advantage of this ability by releasing the drug whenit encounters a specific pH. Another specific triggering mechanism is based on the redox potential. One of the side effects of tumors is hypoxia, which alters the redox potential in the

Physical targeting – Refers to a delivery system that releases the drug only when exposed to a specific microenvironment such as change in pH or environment or the use of external magneticfield.[8]

Drug delivery vehicles

Drug delivery vehicles are at the most important entity required for successful transportation of the loadeddrug at the specific site.

Characteristics of an ideal drug vehicle

An ideal drug vehicle should be able to cross blood brain barriers. It must be recognized by the target cells specifically and selectively. The drug vehicle used should be non-toxic, nonimmunogenic and biodegradable. After recognition, the carrier system should release the drug moiety inside the target organs, tissues or cells.[1]

- **Liposomes**
- **Monoclonal antibodies and fragments**
- **Modified (Plasma) proteins**
- **Quantum dots**

- **Microspheres and Nanoparticles**
- **Lipoproteins**

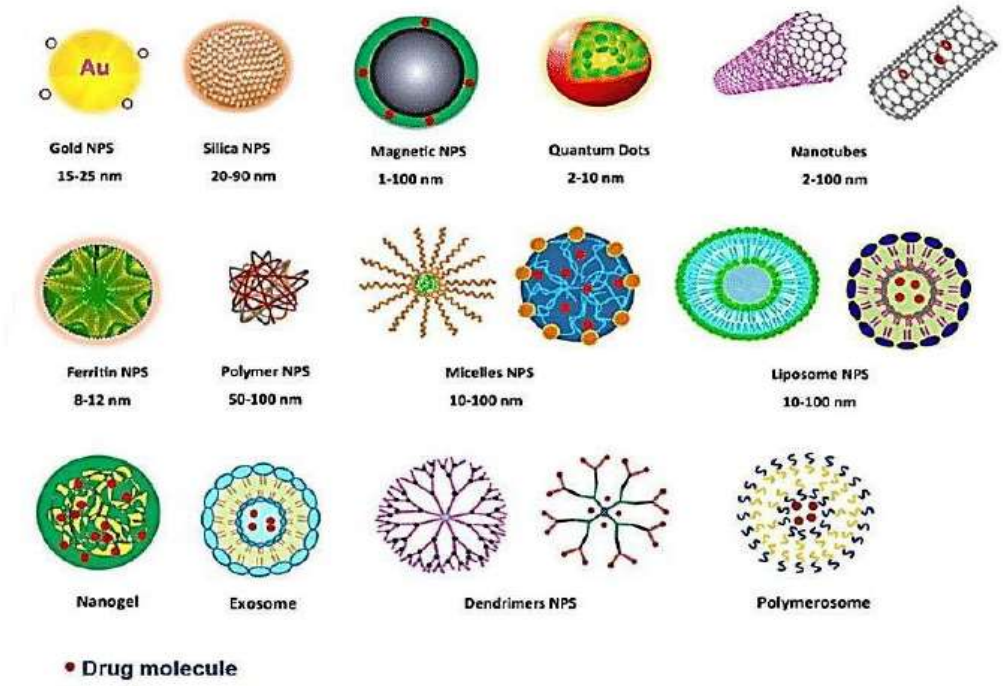


Fig. 4: Targeted drug delivery carriers.

It appears to be about the growing use of monoclonal antibodies as a therapeutic treatment for various ailments including cancer, cardiovascular diseases, and viral infections. These antibodies have become a commercially viable drug and have been combined with other substances to augment their cytotoxic effects. Adalimumab (HUMIRA) is the first human monoclonal antibodyto be approved for use in humans.

Modified plasma proteins

Modified plasma proteins can be intelligent drug vehicle for drug transportation due to their solubility and having relatively small molecular weight. They can easily be modified by the attachment of different molecules like peptides, sugar and other ligands to transport the drug of interest makes them a suitable mode of drug delivery. In the case of liver cell targeting, extensive modification of protein backbones such as albumin have been carried out effective delivery of the drug.[3]

Lipoproteins

There are four main types of lipoproteins that play different roles in transporting lipids throughout the body: 1. Chylomicrons: These are the largest and least dense lipoproteins that carry dietary triglycerides from the intestines to other parts of the body. 2. Very low-density lipoproteins (VLDL):VLDLs are produced by the liver and transport triglycerides synthesized in the liver to various tissues. 3. Low-density lipoproteins (LDL): LDLs, also known as "bad cholesterol", transport cholesterol from the liver to cells in the body. High levels of LDL cholesterol can increase the risk of cardiovascular diseases by leading to the accumulation of cholesterol in the arteries. 4. High- density lipoproteins (HDL): HDLs, often referred to as "good cholesterol," help transport cholesterol away from cells and back to the liver for excretion or recycling. Higher levels of HDL cholesterol are associated with a reduced risk of cardiovascular diseases.

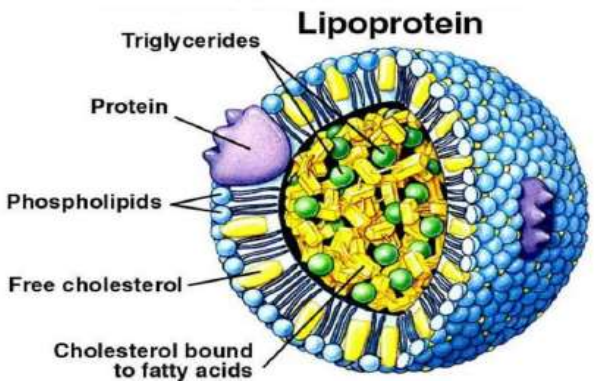


Fig. 5: structure of lipoproteins.

Site-specific targeting is a method used to deliver a larger fraction of a drug to the desired (diseased) site, while reducing the drug's exposure to normal tissues. Encapsulating drugs in liposomes can be used for both active and passive targeting, resulting in a safer and more efficacious therapy. Liposomes are microscopic vesicles composed of one or more lipid layers and are considered to be one of the most successful drug delivery systems. They offer various advantages, such as biocompatibility, high stability in biological fluids, and controlled and prolonged drug release kinetics. One of the most important novel drug delivery systems, liposomes are colloidal particles formed when a phospholipid is hydrated in the presence of water. They range in size from 0.01-0.5 μm in diameter and are biocompatible and biodegradable. Liposomes consist of an aqueous core entrapped by one or more phospholipid bilayers composed of natural or synthetic phospholipids. They are non-toxic, non-immunogenic, and biologically inert and can transport both hydrophilic and lipophilic drugs.

Surface modification can make them more localized to target disease tissue, allowing for drug targeting. Liposomes can act as a carrier for both hydrophilic and lipophilic drugs due to their biphasic environment. Highly hydrophilic drugs (log P <-0.3) are located exclusively in an aqueous domain, whereas lipophilic drugs (log P>5) are entrapped within the lipid bilayer of the liposomes. Drugs with intermediary partition coefficients can also be transported in liposomes. Advanced drug delivery has gained interest in the use of liposomes as a carrier because they mimic biological membranes, making them versatile for study in various fields.

Component of liposomes

Liposomes are spherical vesicles composed of one or more lipid bilayers. They are often used in pharmaceutical and cosmetic industries for drug delivery, as well as in research for various applications. Liposomes can encapsulate both hydrophilic (Water-soluble) and hydrophobic (Fat-soluble) substances.[13]

The main components of liposomes include

- ✓ **Lipid Bilayer:** The core structure of liposomes is the lipid bilayer. This consists of phospholipids, which have hydrophilic (Water-attracting) "heads" and hydrophobic (Water-repelling) "tails." These phospholipids arrange themselves in a bilayer with their hydrophilic heads facing outward and their hydrophobic tails facing inward.
- ✓ **Cholesterol:** Cholesterol is often added toliposome.
- ✓ **Aqueous core:** Liposomes have an aqueous (Water-filled) core that can encapsulate hydrophilic substances, such as drugs, enzymes, or other bioactive molecules.
- ✓ **Encapsulated substance:** Liposomes can encapsulate a wide range of substances, including drugs, peptides, proteins, and nucleic acids. Hydrophilic substances are encapsulated within the aqueous core, while hydrophobic substances can be embedded within the lipid bilayer.
- ✓ **Surface modifications:** Liposomes' surfaces can be modified with various molecules to control their properties, such as targeting specific cells or tissues. These modifications can include ligands, antibodies, or other molecules that help enhance their interaction with target cells.
- ✓ **Size and Charge variability:** Liposomes come in various sizes, ranging from tens of nanometers to micrometer in diameter. Their surface charge can also be modified by incorporating charged lipids, influencing their interactions with biological systems.

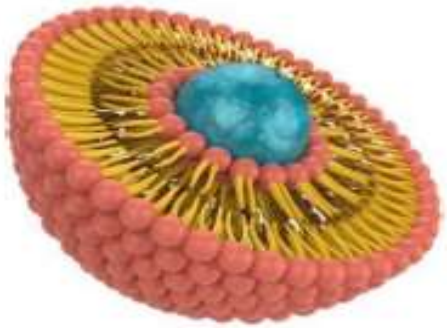


Fig. 6: Basic component.

- ✓ **Buffer or Medium:** Liposomes are typically suspended in an appropriate buffer or medium, which helps maintain their stability and prevents aggregation.
- ✓ **Stabilizers:** Some liposome formulations may include stabilizers to prevent aggregation and fusion of liposomes. Stabilizers can help improve the shelf life and overall stability of liposomepreparations.[13]

Liposomes can be tailored for specific applications by varying their composition, size, surface modifications, and encapsulated substances. They are used in drug delivery to improve the bioavailability and targeted delivery of therapeutic agents, and they also have applications in cosmetics, diagnostics, and research.

Lipids and Phospholipids used for liposomes

Structurally, liposomes are spherical or multilayered spherical vesicles made by the self-assembly of diacyl-chain phospholipids (lipid bilayer) in aqueous solutions. The bilayer phospholipid membrane has a hydrophobic tail and a hydrophilic head that leads to the formation of an amphiphilic structure. Liposomes can be made from both natural and synthetic phospholipids. Lipid composition strongly affects liposome characteristics that include: particle size, rigidity, fluidity, stability, and electrical charge. For example, liposomes formulated from natural unsaturated phosphatidylcholine, as egg or soybean phosphatidylcholine, provide highly permeable and low stable properties. Though, saturated-phospholipids-based liposomes such as dipalmitoyl phosphatidylcholine led to rigid and almost impermeable bilayer structures.[15]

The hydrophilic group in the lipids may be negatively, positively charged, or zwitterionic (bothnegative and positive charge in the same molecule). The charge of the hydrophilic group

provides stability through electrostatic repels. The hydrophobic group of lipids varies in the acyl chain length, symmetry, and saturation.

Cholesterols used for liposomes

Incorporation of sterols in liposome bilayer can bring about major changes in the preparation of these membranes. Cholesterol does not by itself form bilayer structure, i it acts as fluidity buffer. It inserts into membrane with hydroxyl group oriented towards aqueous surface & aliphatic chain aligned parallel to acyl chains in the centre of bilayer. It can be incorporated into phospholipid membranes in very high Conc. upto 1:1 or even 2:1 molar ratios of PC. eliminates the normal electrostatic and hydrogen-bonding interactions.[14]

Cholesterol incorporation increases the separation between the choline head groups and Liposomes are versatile molecules and can be classified in several ways based on their diversity and structural properties, such as composition, shape, size, and surface properties.

Based on their size, they are classified as

- **Unilamellar liposomes:** They are spherical vesicles confined by a single bilayer of an amphiphilic lipid or a combination of such lipids. And they contain an aqueous solution in thecentre of the vesicle.

- **Multilamellar liposomes:** They have an onion-like structure and are made by numerous unilamellar vesicles, forming on the inside of other unilamellar vesicles with smallersizes. Thus, it forms a multilamellar structure of concentric phospholipid spheres separated by water layers.[16]

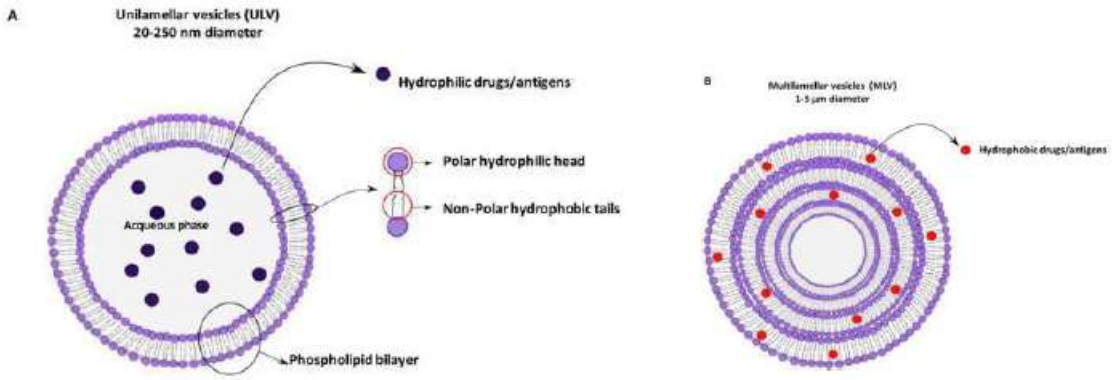


Fig. 7: Unilamilar and Multi lamellar liposomes.

Based on their structural parameters, they are categorized into

- **Multilamellar liposomes/vesicles (MLV):** They consist of more than five multiple lipid bilayers, forming a multilamellar vesicle.
- **Oligolamellar vesicles (OLV):** Vesicles containing concentric lamellae, ranging between two to five, are called oligolamellar vesicles.
- **Multivesicular liposomes/vesicles (MVV):** They are also known as vesosomes meaning "*vesicles-inside-vesicles*." In an aqueous solution, they contain internal, non-concentrically arranged smaller vesicles made from naturally occurring or synthetic bilayer-forming amphiphiles.[17]

Liposomes are synthesized using an extensive range of methods. Based onsynthesis, they are classified as

- **Dehydration and rehydration (DRV):** In this process, the small unilamellar vesicles containing the buffer are dried and rehydrated with the aqueous solution, containing the compound needed to be incorporated in This method is generally used to form anoligolamellar vesicle.[18]

- **Reverse phase evaporation (REV):** This technique is based on the formation of invertedmicelles. They are formed after sonicating a mixture of a buffered aqueous phase (containing the water-soluble molecules to be encapsulated into the liposomes) and an organic phase in which the amphiphilic molecules are solubilized.[19]

The organic solvent is slowly eliminated, converting the inverted micelles into viscous or gel forms. Then the gel state collapses at a critical stage, disrupting some of the inverted micelles. Too many phospholipidsin the environment cause a whole bilayer to develop around the remaining micelles, resulting in liposome formation.[19]

- **Extrusion technique (VET):** Here, multilamellar vesicles are pushed through a polycarbonate membrane filter containing pores of the desired size to control the vesicle size distribution.[18]

- **Freeze and Thaw extrusion method (FAT):** In this method, liposomes formed using the thin-film method are vortexed with the material needed to be incorporate in the vesicles. This is done until the whole lipid film is suspended. Then, the resulting vesicleare frozen in warm water and vortexed again.[18]

- **Sonication:** It's one of the most extensively used techniques for liposome preparation. Here, multilamellar vesicles formed by other preparation techniques are subjected to sonication, resulting in small unilamellar vesicles.

- **Solvent dispersion method:** It includes ether injection and ethanol injectionmethods.[18]

❖ **Ether injection method:** Here, a lipid solution mixed with diethyl ether or an ether-methanol mixture is progressively injected into an aqueous solution, containing the substance to be encapsulated, at 55°C to 65°C or under reduced pressure. Then, ether is eliminated under a vacuum that leads to the formation of liposomes.[19]

❖ **Ethanol injection method:** Here, a lipid solution dissolved in ethanol is rapidly injected into a large buffer volume, resulting in the rapid formation of multilamellar liposomes/vesicles.[19]

- **Thin-film hydration method:** This is the most common and widely used technique. In this method, the lipids are dissolved in an organic solvent, like chloroform or mixtures of chloroform and methanol. Then, the solvent is removed by film deposition under vacuum.[18]

The organic solvent is completely evaporated, and the lipid residues are again hydrated using an aqueous buffer, resulting in the swelling and hydration of lipids. These lipids lead to the formation of liposomes. Liposomes produced using this technique are an aggregation of multilamellar vesicles having different sizes.[18]

Liposomes can also be classified depending on their composition as

- **Conventional liposomes:** They are the first liposomes created and used in pharmaceutical applications. They consist of natural lipids or phospholipids, like 1, 2-distearoyl-sn-glycero-3-phosphatidylcholine (DSPC), sphingomyelin, egg phosphatidylcholine,and monosialoganglioside.[20]

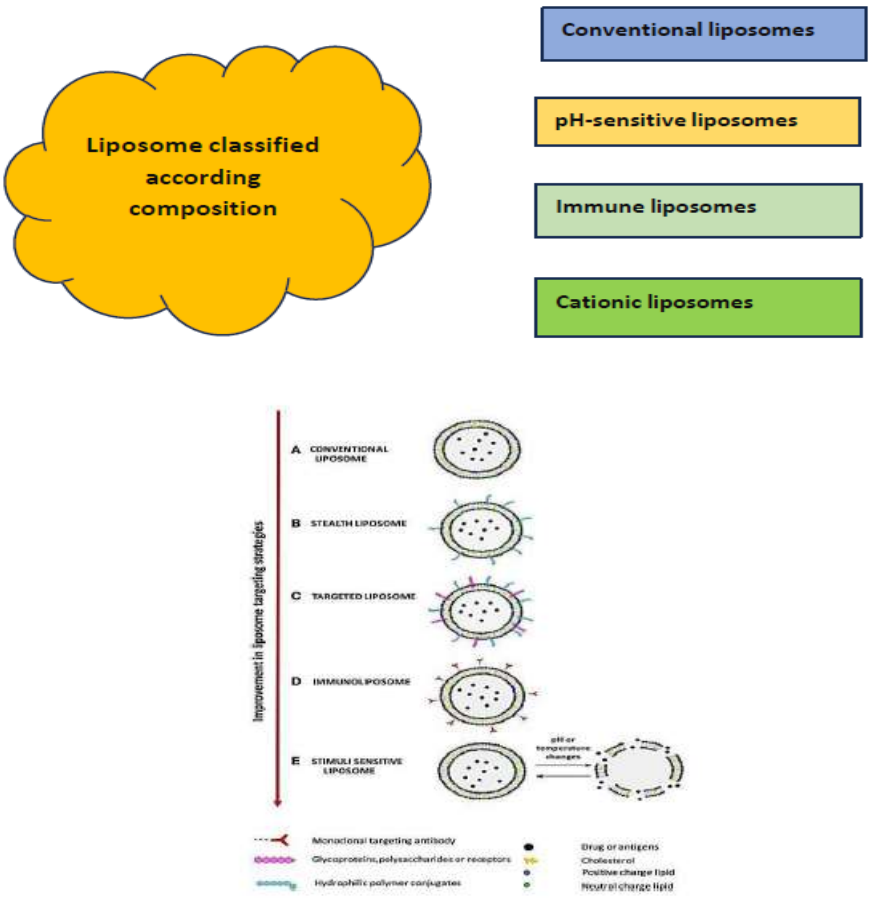
- **pH-sensitive liposomes:** Their design is inspired by viruses that merge with endosomal membranes and deliver their genetic material to the cytosol before reaching the lysosomes.

They generally consist of phosphatidylethanolamine and titratable stabilizing amphiphilic molecules that are unstable under acidic conditions.[21]

- **Cationic liposomes:** They are formed by mixing the cationic lipids, like 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), with DNA. The complex form is due to the interaction between the positive head group of the lipid with the negative phosphate group of DNA.[20]

- **Immune liposomes:** These liposomes contain monoclonal antibodies or their fragments with phospholipids. In some cases, the antigens are reconstituted into liposomesmembrane or inserted into the interior core of the liposome to enhance immune response.[20]

- **Thin-film hydration method:** These are designed to extend the circulation time of liposomes in blood. Maximum circulation time is achieved by using polyethylene glycol covalently bound to the phospholipid. They are a perfect fit to improve tissue localization.[20]



Pic 8: An illustration of different types of liposomes based on their Composition and Targeting strategie.

Advantages and Limitaions

Advantages

- ***Biocompatibility:*** Liposomes are composed of natural lipids, making them biocompatible and well- tolerated in biological systems. This feature reduces the risk of adverse reactions and toxicity.

- ***Encapsulation of Hydrophilic and Hydrophobic compounds:*** Liposomes can encapsulate a wide range of compounds, including hydrophilic (Water-soluble) and hydrophobic (water- insoluble) drugs, making them versatile delivery vehicles.

- ***Improved solubility:*** Liposomes can enhance the solubility of poorly water-soluble drugs, which can improve their bioavailability.

- ***Targeted drug delivery:*** Surface modification of liposomes allows for specific targeting of drugs to particular tissues or cells, reducing off-target effects and enhancing therapeutic efficacy.

- ***Controlled release:*** Liposomes can be designed to release their contents gradually, providing sustained drug release over time. This is advantageous for maintaining therapeutic drug levels.

- ***Protection of labile compounds:*** Liposomes can protect sensitive or labile compounds, such as proteins or nucleic acids, from degradation by enzymes or environmental factors.

- ***Reduced toxicity:*** Liposomal drug delivery can help reduce the toxicity of certain drugs by minimizing their exposure to healthy tissues and cells.

- ***Improved pharmacokinetics:*** Liposomes can extend the circulation time of drugs in the bloodstream, allowing for a more prolonged therapeutic effect.

- ***Enhanced cellular uptake:*** Liposomes can be designed to facilitate cellular uptake, improvingthe delivery of drugs or genetic material to target cells.

- ***Versatility:*** Liposomes can be tailored to meet the specific requirements of different applications, including various lipid compositions, sizes, and surface modifications.

- ***Applications in cosmetics*****:** Liposomes are used in cosmetics for the delivery of active

ingredients, such as vitamins and antioxidants, to the skin, improving their effectiveness.

- ***Research tools:*** Liposomes are valuable tools in laboratories for studying membrane interactions, drug release kinetics, and cellular uptake mechanisms.

- ***Biological mimicry:*** Liposomes can mimic biological membranes, making them suitable for studying cell-membrane interactions and for modeling biological processes.

- ***Immunotherapy:*** Liposomes can be utilized in vaccine delivery, enhancing the immune response and improving vaccine efficacy.

Limitaions

- ***Size and Homogeneity:*** It can be challenging to produce liposomes of uniform size and shape, which can impact their stability and drug delivery efficiency. Variability in liposome sizecan lead to inconsistent results.

- ***Stability:*** Liposomes can be sensitive to environmental factors like temperature, pH, and mechanical stress. They can easily fuse or leak their contents, making them less stable for storage and in vivo applications.

- ***Limited loading capacity:*** The encapsulation efficiency of liposomes for certain drugs or molecules can be limited. This can be a significant drawback if high drug payloads are required.

- ***Short Circulation Half-Life:*** In the bloodstream, liposomes can be rapidly removed by the reticuloendothelial system (RES), reducing their circulation time. Prolonged circulation is essential for drug delivery to specific target sites.

- ***Leakage:*** Over time, liposomes can release their payload prematurely, which reducestheir effectiveness in drug delivery. This leakage can be influenced by factors like lipid composition and cargo encapsulation.

- ***Biocompatibility and Immunogenicity:*** Liposomes may trigger immune responses in some individuals, causing potential adverse reactions. It is essential to consider the liposomecomposition and the route of administration to mitigate this issue.

- ***Limited targeting ability:*** While liposomes can be modified to target specific tissues or

cells, their targeting efficiency may not be as high as other drug delivery systems, like nanoparticles or antibodies.

- ***Scale-Up Challenges:*** Scaling up liposome production can be complex and expensive. Ensuring consistent quality and batch-to-batch reproducibility can be challenging.

- ***Regulatory approval:*** Regulatory approval for liposomal drug products can be more challenging to obtain due to the need to demonstrate the safety and efficacy of both the drugand the liposome carrier.

- ***Cost:*** The production of liposomes can be expensive due to the cost of high-quality lipids, specialized equipment, and complex manufacturing processes.

- ***Limited applications:*** Liposomes may not be suitable for all types of drugs or therapies. The choice of drug and disease target must align with the capabilities of liposomal drug delivery.[22]

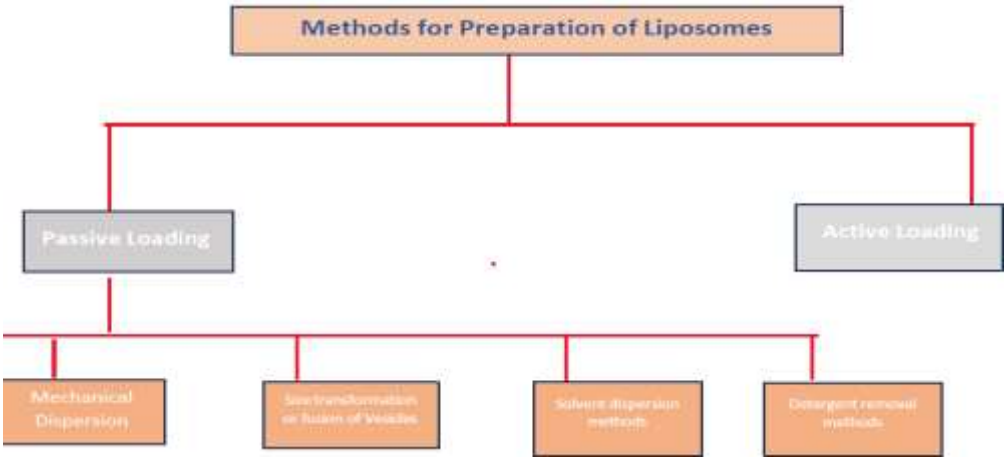
Preprations of liposomes

A basic understanding of the physicochemical properties of phospholipids is needed to understand the liposome formation. Phospholipids are amphiphilic (having both aqueous and polar moiety affinity), it has two fatty acid chains containing 10-24 carbon atoms and 0-6 double bond in each chain, which are the non-polar tail of phospholipids. The polar end is mainlyphosphoric acid bond to the water-soluble molecule when phospholipids are hydrated, they are arranged in such an orientation that the polar portion of the phospholipids remain in contact with the polar environment and at the same time shield the non-polar part. The most common natural polar phospholipids are phosphatidylcholine (PC).[23]

❖ **Selection of methods for liposome preparation**

✓ The correct choice of liposome preparation method depends on the following parameters:
✓ The physicochemical characteristics of the material to be entrapped and those of the liposmesingredien
✓ The nature of the medium in which the lipid vesicles are dispersed;
✓ The effective concentration of the entrapped substance and its potential toxicity;
✓ Additional processes involved during application/delivery of the vesicles;
✓ Optimum size, polydispersity and shelf-life of the vesicles for the intended application and
✓ Batch-to-batch reproducibility and possibility of large-scale production of safe and

efficient liposomal products.[23]



Detailed explanation of each preparation methods

- **Passive loading**

This loading technique is to load or encapsulate drug molecules before forming or during preparing liposomes. During liposomes preparation when the lipid film dissolved in drug containing aqueous buffer then that hydrophilic or water-soluble drugs is loaded at the centre of liposome vesicle. When lipophilic drugs added to lipid phase of liposome components then that lipophilic drug will load in between lipid bilayers. The unentrapped drug is removed using gel-filtration chromatography or dialysis for liposomal dispersion.

- **Active loading**

Certain compounds which have both aqueous and lipid solubility and having ionisable groups can be loaded after formation of vesicles. This type of method is called remote or active loading of drug molecules. In this remote or active loading several methods exist in preparing of liposomes. Doxil™ is one of the liposomal products prepared by this method.[24]

Mechanical dispersion method

✓ **Lipid film hydration method:** Thin film hydration is the earliest technique for preparing liposomes. The preparation of liposomes in this method was conducted by three processes.

✓ **Preparation of lipid for hydration:** The lipids must first be dissolved and mixed in an or chloroform: Methanol mixtures) to assure a homogeneous mixture of lipids. Once the lipids are thoroughly mixed in the organic solvent, the solvent is removed to yield a lipid film. For small volumes of organic solvent (<1mL), the solvent may be evaporated using a dry nitrogen or argon stream in a fume hood. For film on the sides of a round bottom flask.

The lipid film is thoroughly dried to remove residualorganic solvent by placing the vial or flask on a vacuum pump overnight. The lipid solution istransferred to containers and frozen by placing the containers on a block of dry ice. After freezing completely, the frozen lipid cake is placed on a vacuum pump and lyophilized until dry (1-3 days depending on volume). The thickness of the lipid cake should not be more than the diameter of the container being used for lyophilization. Dry lipid films or cakes can be removed from the vacuum pump, the container should be closed tightly and taped, and stored frozen until ready to hydration.

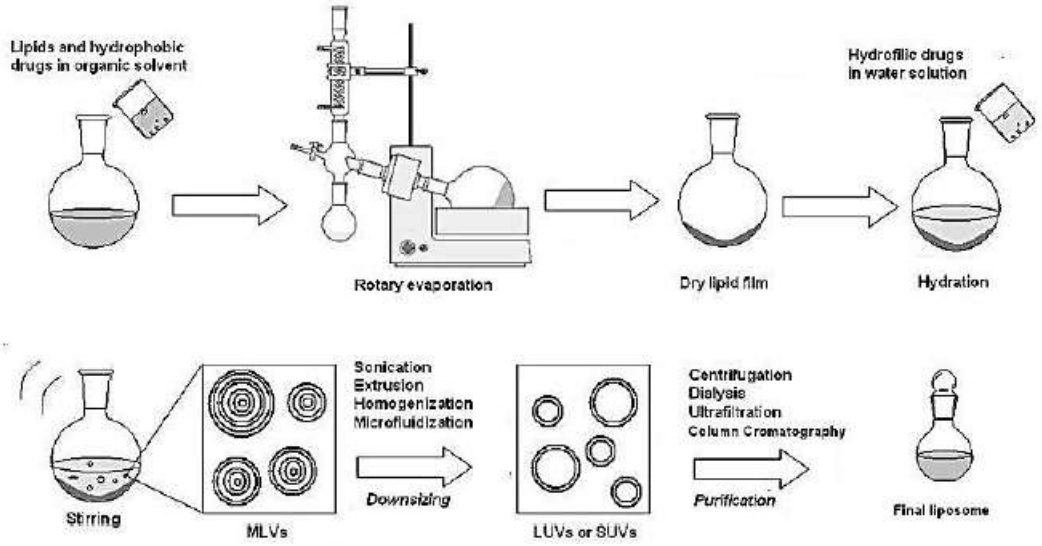


Fig. 9: Preparation of lipid hydration.

- **Hydration of lipid film:** Hydration of the dry lipid film/cake is accomplished simply by adding an aqueous medium to the container of dry lipid and agitating. The temperature of the hydrating medium should be above the gelliquid crystal transition temperature (Tc or Tm) of the lipid. For high transition lipids, this is easily accomplished by transferring the lipid suspension to a round bottom flask and placing the flask on a rotary evaporation system without a vacuum. Spinning the round bottom flask in the warm water bath maintained at a temperature above the Tc of the lipid suspension allows the lipid to hydrate in its fluid phase with adequate agitation. The product of hydration is a large, multilamellar vesicle (LMV) analogous in structure to an onion, with each lipid bilayer separated by a water layer. Now, the particles can be downsized by a variety of techniques, including sonication or extrusion.[25-27]

- **Sizing of lipid suspension:** Disruption of LMV suspensions using sonic energy

(Sonication) typically produces small, unilamellar vesicles (SUV) with diameters in the range of 15-50nm. The most common instrumentation for preparation of sonicated particles are bath and probe tip energy input to the lipid suspension but suffer from overheating of the lipid suspension causing degradation. Sonication of an LMV dispersion is accomplished by placing a test tube containing the suspension in a bath sonicator (or placing the tip of the sonicator in the test tube) and sonicating for 5-10 minutes above the Tc of the lipid. Mean size and distribution are influenced by composition and concentration, temperature, sonication time and power, volume, and sonicator tuning.[27]

- ✓ **Microemulsification method:** A method based on microfluidization i.e., microemulsification is used for the large-scale manufacture of liposomes. The preparation of antibiotic liposomes by thin-layer hydration method followed by sonication with a bath-typesonicator and microfluidization in order to achieve partial homogenization. The process of microfluidization is reproducible and yield liposomes with good aqueous phase encapsulation.[28]

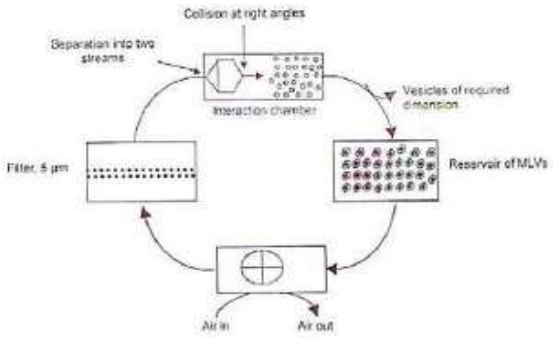


Fig. 10: Micro emulsification.

- ✓ **Sonication:** This is the most widely used method for the preparation of SUV from MLV, prepared from thehandshaking method and rotary evaporator method.There are two types of sonication methods used in the preparation of SUVs.[29]

- ✓ **Probe sonication method:** In this method, the tip of the titanium probe is directly dispersed into liposome dispersion for the production of SUVs. In this method, the energy input is highcontrolling heat, liposome dispersion is kept in the ice bath. The main disadvantage of this method is that the titanium fragment is sludge in a solution and contaminate it.

✓ **Bath sonication:** In this method, liposome dispersion in a container is placed on the sonication bath. This method is more convenient as compared to probe sonication for the production of SUVs because titanium contamination.

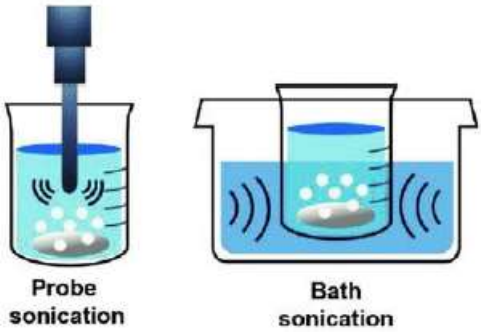


Fig. 10: Bath sonication.

- **Membrane extrusion method:** Extrusion is a method in which liposome suspension is passed, through a film filter with defined pore size. Extrusion method needs an extruder carrying with a pump that pushes suspensions through the films to accomplish the extrusion process. Extrusion is widely used in sizing the liposomes. This technique can be used to prepare nanosized liposomes.[31]

- **Dried reconstituted vehicles:** Dried reconstituted vesicles (DRV) are liposomes that are formulated under mild conditions and have the capability to entrap substantially high amounts of hydrophilic solutes (Compared with other types of liposomes). These characteristics make this liposome type ideal for entrapment of labile substances, as peptide, protein, or DNA vaccines, or in general biopharmaceuticals and sensitive drugs.[32]

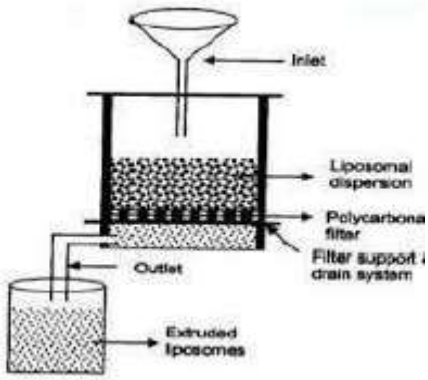


Fig. 11: Membrance extraction.

- **Dehydration-Rehydration method:** Dehydration and rehydration methods are alternative approaches to traditional lipid film hydration for liposome preparation.

These methods can provide advantages in terms of controlling liposome size, encapsulation efficiency, and stability.

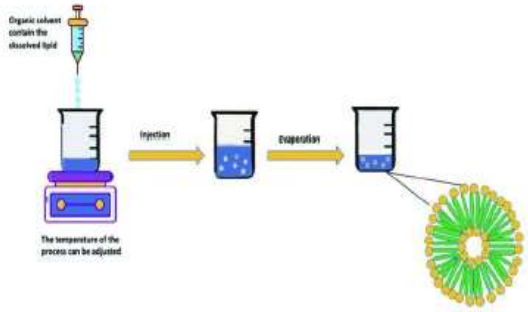
- **Dehydration:** In this method, lipids are first hydrated in an organic solvent to create a lipid dispersion. This dispersion is then rapidly injected into an excess of an aqueous solution.

- **Rehydration:** The rapid injection of the lipid dispersion into the aqueous solution leads to the solvent conditions, forcing the lipids to self-assemble into liposomes. This method is often used to create unilamellar liposomes with a more homogeneous size distribution compared to traditional.

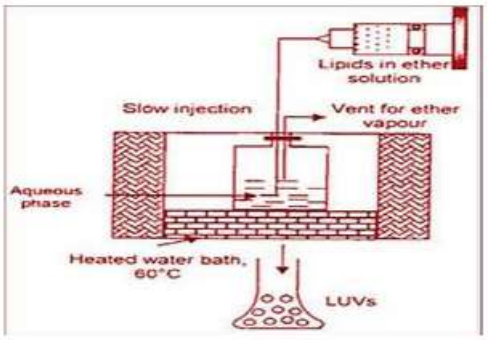
- **Freeze-thawed liposomes:** SUVs are rapidly frozen and thawed slowly. The short-lived sonication disperses aggregated materials to LUV. The creation of unilamellar vesicles is as a result of the fusion of SUV throughout the processes of freezing and thawing. This type of synthesis is strongly inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium. The encapsulation efficacies from 20% to 30% were obtained.[34]

- **Ethanol injection:** To a buffer a solution of lipid and ethanol is injected, resulting in the formation of MLVs. The drawback is the formation of a heterogeneous population of liposomes (30-110 nm). It is also difficult to remove ethanol from a solution consequently increasing the chances for the inactivation of biologically active macromolecules.[35]

- **Ether injection:** In this method, lipid dissolved in a diethyl-ether or ether-methanol mixture is gradually injected in an aqueous medium containing drug at the temperature of 50 to 65^{o}c or reduced pressure. The removal of ether under vacuum results formation of liposomes. The main drawback of this technique is the formation of a heterogeneous population of liposomes (70-200 nm) and exposure of liposomes in high temperatures during encapsulation which can hamper the stability of liposomes.[35]



Pic 12: Ethanol injection metho.



Pic 13: Ether injection.

- **Double emulsion vesicles:** The double emulsion vesicle method, also known as the double emulsion solvent evaporation technique, is a specialized approach for preparing liposomes or other vesicular systems, especially when encapsulating hydrophilic substances. The double emulsion method involves creating two layers of emulsions, resulting in a more controlled encapsulation process. Here's how it works.[36]

- **Primary Emulsion (Water-in-Oil, W/O): Inner Aqueous Phase:** The aqueous solution containing the substance to be encapsulated is dispersed within an organic solvent that is immiscible with water. This forms the primary water-in-oil emulsion. The substance to be encapsulated can be a drug, protein, or other hydrophilic compound.

- **Lipid solution:** Lipids are dissolved in the organic solvent, forming a lipid solution.

- **Emulsification:** The inner aqueous phase (containing the substance) is mixed or homogenized with the lipid solution using techniques like ultrasonication or mechanical stirring. This creates small droplets of the inner aqueous phase surrounded by lipids in the organic solvent.

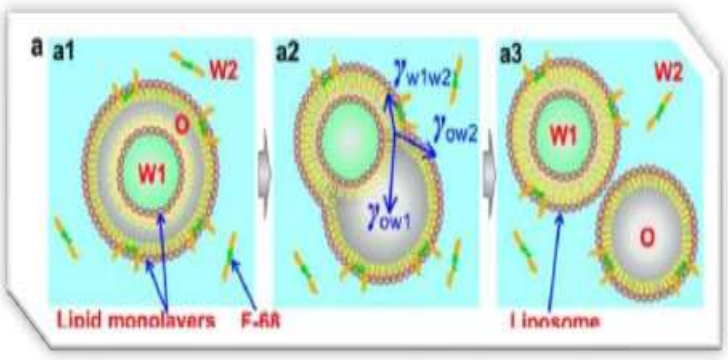


Fig. 14: Double emulsion method.

Preparation flowchart of double emulsion vesicle method

- **Secondary Emulsion (Oil-in-Water-in-Oil, O/W/O): External aqueous phase:** The primary emulsion is then mixed with a larger volume of an external aqueous solution, which acts as the continuous phase. This creates the secondaryoil-in-water emulsion.

- **Emulsification:** The primary emulsion (W/O) is mixed or homogenized with the external aqueous phase, resulting in small droplets of the primary emulsion suspended in the external aqueous phase.

- **Evaporation and Vesicle formation: Evaporation:** The organic solvent present in both the primary and secondary emulsions is then allowed to evaporate over time, leaving behinda lipid bilayer around the encapsulated aqueous droplets.

- **Vesicle formation:** As the organic solvent evaporates, the lipid bilayer forms vesicles or liposomes around the encapsulated aqueous phase, resulting in a water-in-oil-in-water (W/O/W) double emulsion vesicle structure.[36]

- **Reverse evaporation vehicles:** The reverse phase evaporation technique is composed of inverted micelles or water-in-oil emulsions in which the water phase contains the interested drugs and the organic phase consists of the lipids to form liposomal bilayer.[29]

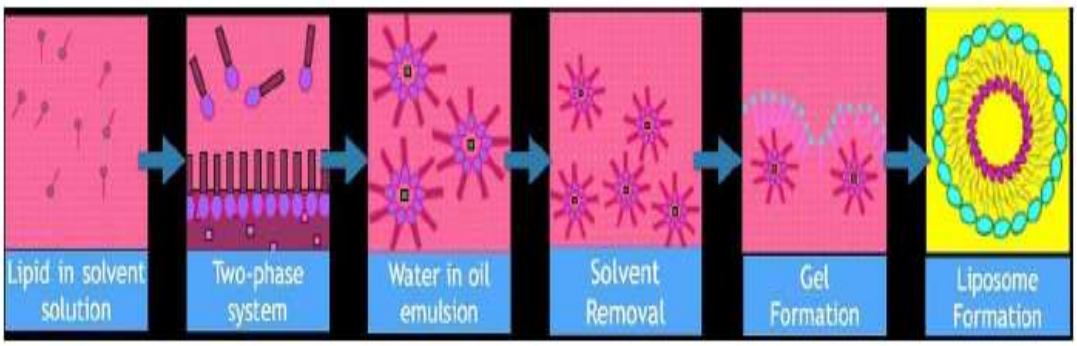


Fig. 15: Reverse phase evaporation method.

- **Stable plurilamellar vesicles:** Stable plurilamellar vesicles (SPLVs), also known as multilamellar vesicles (MLVs), are liposomal structures composed of multiple concentric lipid bilayers. Preparing stable SPLVs involves specific techniques to ensure the successful formation of multiple lipid bilayers. Here's a general overview of how to prepare stable plurilamellar vesicles:

- **Lipid film formation:** Dissolve the desired lipids in an organic solvent (usually chloroform) to

- **Solvent evaporation:** Evaporate the organic solvent using a rotary evaporator or by gently passing a stream of nitrogen gas over the lipid solution. This results in a thin lipid film coating the walls of the flask.

- **Hydration to form multilamellar vesicles:** Add an appropriate aqueous buffer to the lipid-coated flask to vesicles. The hydration process leads to the self-assembly of multiple lipid bilayers.

Optional steps for size reduction

- **Sonication:** To reduce the size of the vesicles and promote uniformity, subject the vesicle suspension to sonication using a probe or bath sonicator.

- **Extrusion:** Pass the vesicle suspension through a series of polycarbonate membranes using an extruder to further reduce vesicle size and enhance uniformity.

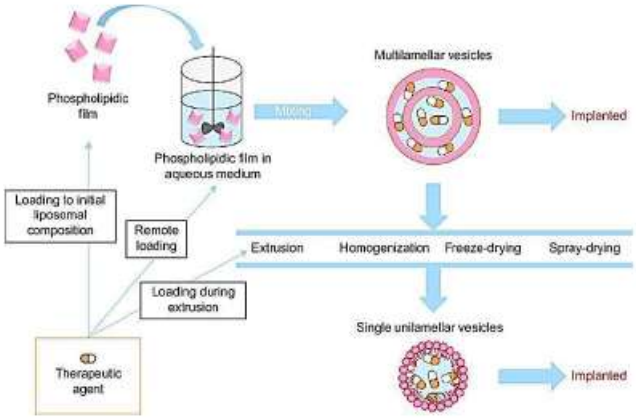


Fig. 15: Stable plurilamellar vesicle.

- **Purification and Characterization:** Centrifuge the vesicle suspension to remove any larger aggregates or unencapsulated material. Characterize the vesicles using techniques like dynamic light scattering (DLS), electron microscopy, and zeta potential measurement to assess size distribution, stability, and surface charge.

- **Detergent removal method:** The detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids. As the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs.[38]

- ***Dialysis*** - The detergents at their critical micelleconcentrations (CMC) have been used to solubilize lipids. As the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. The detergents were removed by dialysis.[34-36]

A commercial device called LipoPrep (Diachema AG, Switzerland), which is a version of dialysis system, is obtainable for the elimination of detergents. The dialysis can be performed indialysis bags engrossed in large detergent free buffers (Equilibrium dialysis).[17]

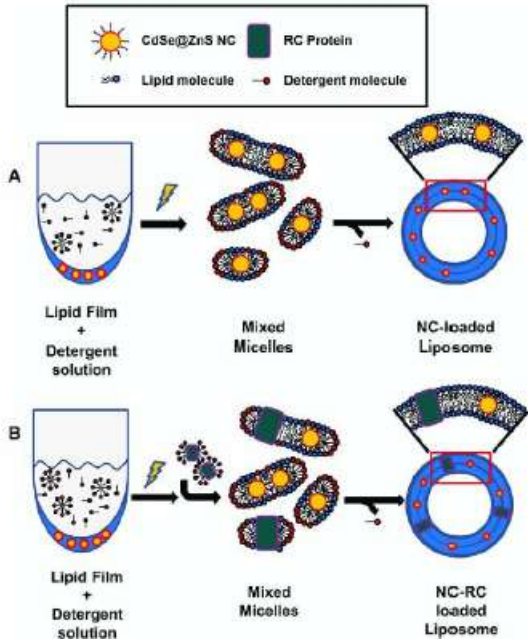


Fig. 16: Stable plurilamellar vesicle.

- ***Gel-permeation chromatography*** - In this method, the detergent is depleted by size special chromatography. Sephadex G-50, Sephadex G-l 00 (Sigma-Aldrich, MO, USA), Sepharose 2B-6B, and Sephacryl S200-S1000 (General Electric Company, Tehran, Iran) can be used for gel filtration. The liposomes do not penetrate into the pores of the beads packed in a column. They percolate through the inter-bead spaces. At slow flowrates, the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; therefore, pre-treatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

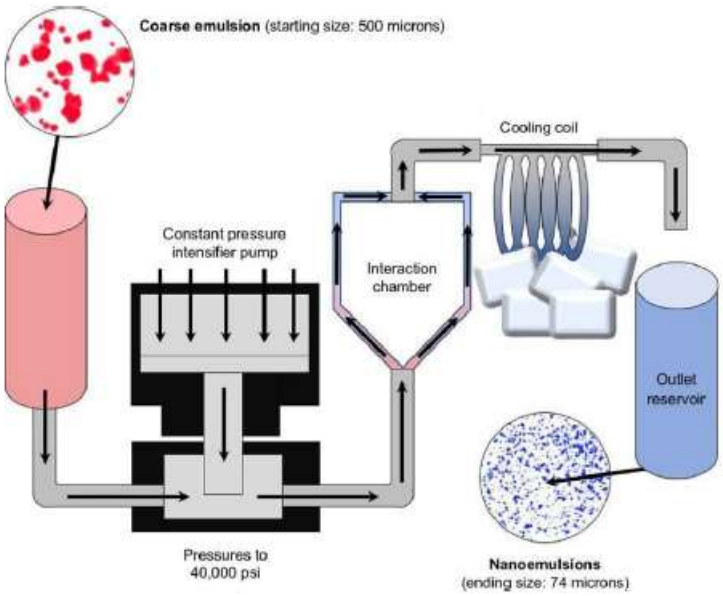
- ***Dilution*** - Upon dilution of aqueous mixed micellar solution of detergent and phospholipids with buffer, the micellar size and the polydispersity increase fundamentally,

and as the system is diluted beyond the mixed micellar phase boundary, a spontaneous transition from polydispersed micelles to vesicles occurs.[30]

Industrial productionof liposomes

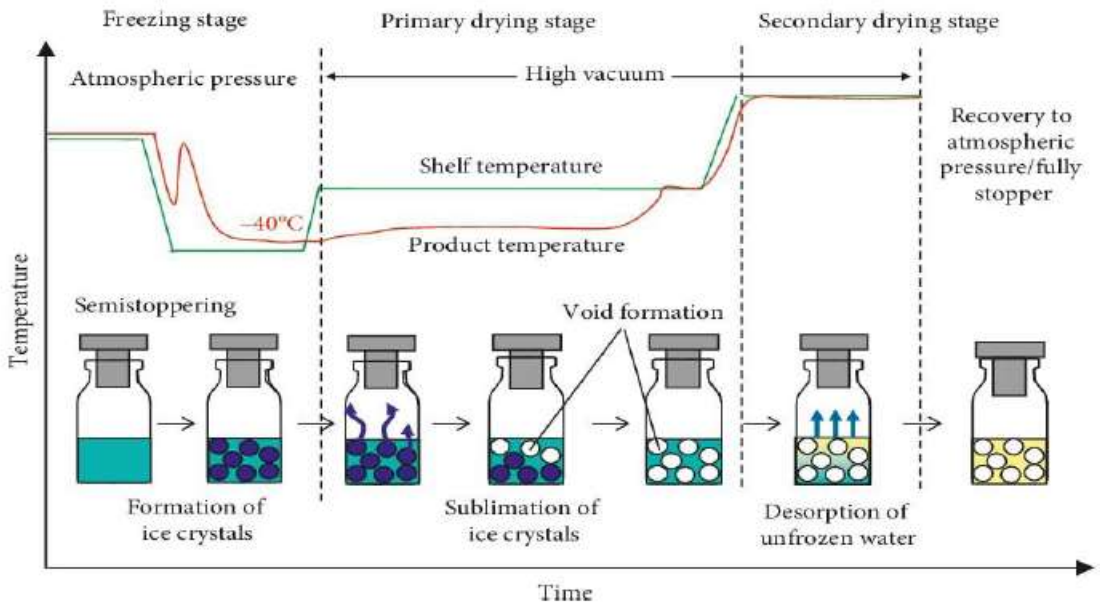
- **Microfluidization method**

Stachowiak also developed a microfluidics method for simultaneously creating and loading giant liposomes based on a pulsed microfluidic jet. In the microfluidic jetting, a planar lipid bilayer is deformed into a vesicle that is filled with the solution from the jet. Compared to other conventional giant liposome production methods, this method can rapidly and controllably generate multiple monodisperse and unilamellar vesicles.[31]



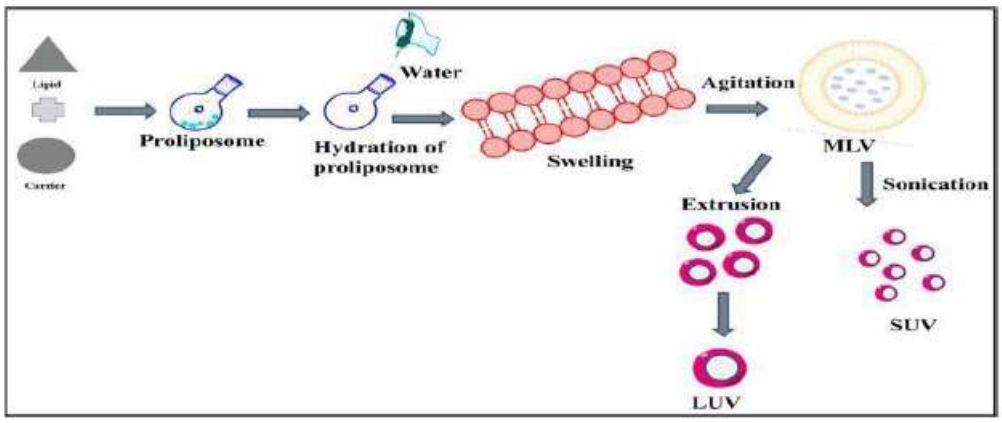
Pic 17: Microfluidization method.

- **Lyophilization:** Freeze-drying (Lyophilization) involves the removal of water from products in the frozen state at extremely low pressures. The process is generally used to dry products that are thermolabile and would be destroyed by heat-drying. The technique has a great potential as a method to solve long term stability problems with respect to liposomal stability. It is exposed thatleakage of entrapped materials may take place during the process of freeze- drying and on reconstitution.[31]



Pic 18: Lyophillization.

- **Proliposomes:** In proliposomes, lipid and drug are coated onto a soluble carrier to form free-flowing granular material which on hydration forms an isotonic liposomal suspension. The proliposome approach may provide an opportunity for cost-effective large scale manufacturing of liposomes containing particularly lipophilic.[32]



- **Heating method:** It is also an organic solvent free technique. In this method, lipids are hydrated directly with aqueous solution, and heated for not less than one hour above the Tm of the used phospholipids in the presence of a 3–5 % hydrating agent as glycerin or propylene glycol. The suspension can be heated up to 100 °C when adding cholesterol to the formulation. The hydrating agents act as a stabilizer and isotonizing additives that prevent nanoparticle coagulation and sedimentation. Moreover. The hydration agents provide a cryoprotective effect that makes the heating method an efficient method for the

formulation of powder inhalable liposomes.[35]

- **Nobel methods of production of liposomes**

Super critical reverse phase evaporation: Briefly, the apparatus consists of three parts: a viewing cell with a variable volume, an HPLC pump for feeding aqueous solution into the viewing cell and a high-pressure pump for CO2 and pressure control by moving the piston in the viewing cell. The ethanol solution of lipid materials is measured with an electronic balance and fed into the cell After the lipid materials are placed in the viewing cell the cell is sealed, and a magnetic tip is used for stirring inside the viewing cell and gaseous CO2 is introduced into the cell.

The temperature is then raised to a chosen value, which could reach both the phase transition temperature of the phospholipids and the supercritical temperature of carbon dioxide. The pressure is also kept above the supercritical value. After several seconds to reach equilibration, an aqueous solution of the model drug is slowly introduced into the cell through the HPLC pump, until a sufficient amount of solution is reached. Finally, the pressure is reduced to release CO2 and a homogeneous liposomal dispersion is formed.[37]

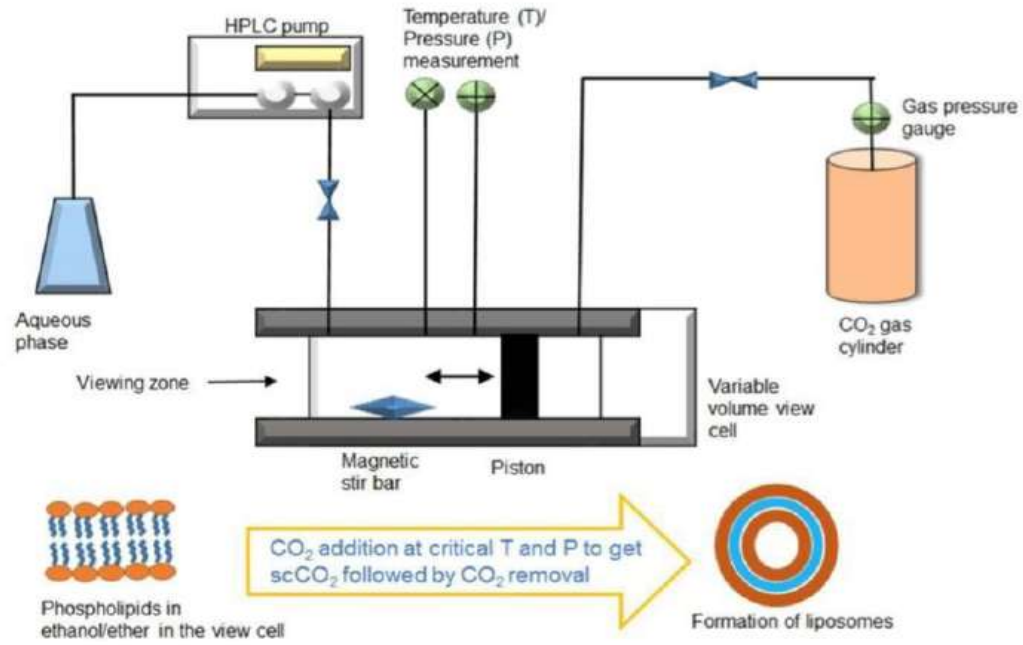


Fig. 18: Super critical reverse phase evaporation.

Supercritical Anti-Solvent (SAS) Method: In this method, a solution containing an organic solvent and the solute (lipids and active drugs) is placed in contact with a SCF, (such as SC-CO2) which is (completely) miscible with the organic solvent but acts as anti-solvent for the

solute. The dissolution of SC- CO2 in the liquid phase and the successive organic solvent extraction favors the precipitation of the lipidic nanoparticles. The processed solution is successively hydrated in an aqueous buffer solution, leading to the formation of liposomes. To remove any organic solvent in excess, a washing stage (with pure CO2) is finally performed.[38]

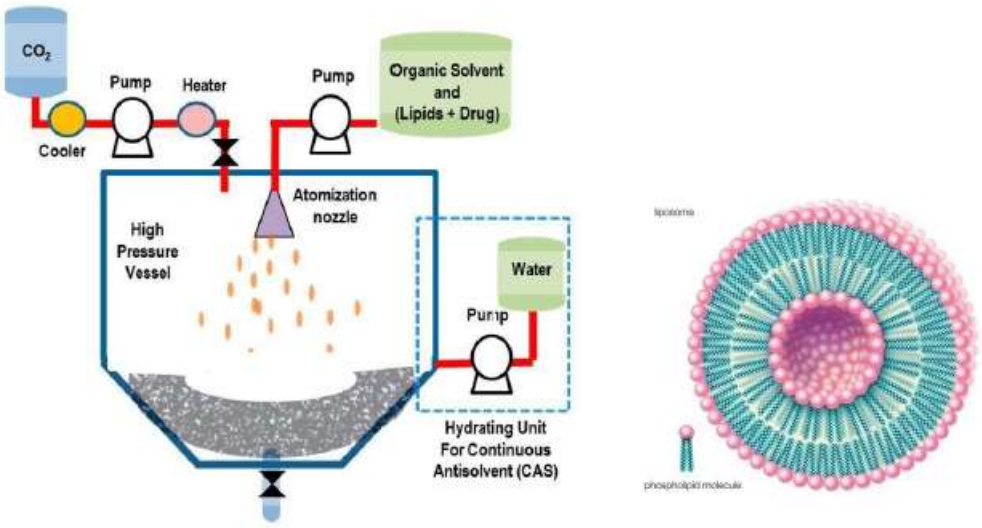


Fig. 19: Preparation Flowchart of SAS Method.

Schematic representation of the SAS method for liposome preparation. The SCF CO2 is pumped to the top of the high-pressure vessel until the system reaches a constant temperature and pressure. Subsequently, an organic solution containing the lipids and active substance is sprayed (through an atomization nozzle) as fine droplets into the above SCF bulk phase. Liposomes are finally formed in a successive hydration step. In the continuous antisolvent (CAS) method, the addition of the hydration unit allows for the hydration of the lipid suspension in the same autoclave under pressure.[38]

- **Rapid Expansion of a Supercritical Solution (RESS) Method:** The RESS technique is carried out in two steps: Initially, a solution containing lipids is dissolved in ethanol (5–10% of *v*/*v*) and supercritical CO2 within an extractor. The resulting solution is depressurized through a heated nozzle in a low-pressure chamber. The rapid expansion/decompression (At supersonic speeds) through a nozzle favors the decrease of the pressure and the evaporation of CO2, thus leading to the supersaturation, and then, to the precipitation of the solid (That will be collected from the gaseous stream.

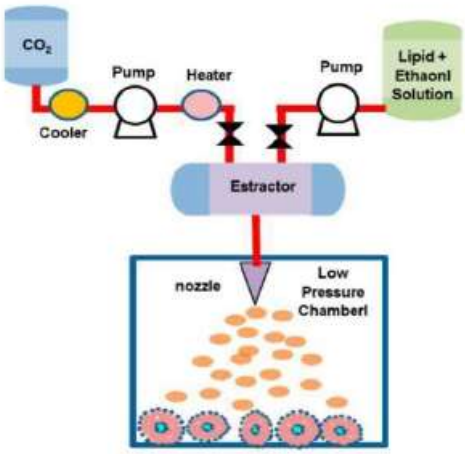


Fig. 20: Preparation of RESS method.

- **Supercritical-Assisted Liposome Formation (SuperLip) Method:** In the SuperLip method, the ethanol solution and CO2 are continuously fed to a homogenizer, forming an expanded liquid which is then delivered to a precipitation vessel. In this vessel, water with droplets containing drugs are produced by means of an atomization process (inside a high-pressure vessel). The droplets are then surrounded by a lipid layer (thus favoring the formationof w/CO2 emulsion) which falls into the water pool placed at the bottom of the vessel, where liposomes are formed.[38]

A high-pressure vessel is filled with an expanded liquid mixture (Formed by PLs/ethanol/CO2 containing drugs). Water droplets are produced by atomization inside a high- pressure vessel. These droplets are rapidly surrounded by a lipid layer, forming a w/CO2.

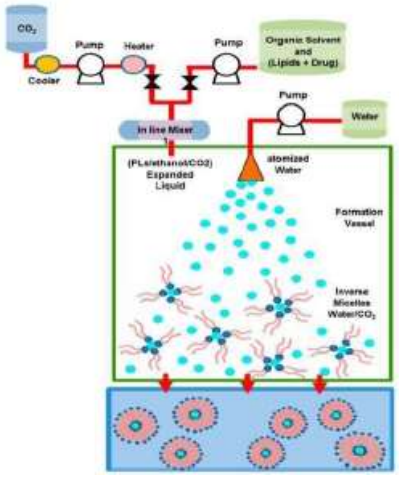


Fig. 21: Preparation of super lip method.

Depressurization of an Expanded Liquid Organic Solution into Aqueous Suspension (DELOS) Method: In the DELOS method, the lipids (and active drugs) are first dissolved in an organic solvent (such as ethanol) contained in a vessel at fixed temperature and pressure, and then mixed with SC- CO2 (used as a co-solvent). The mixture (depressurized at 35–55 bar) is then expanded into CO2, and (through a nozzle) is successively injected in a vessel containing water bath and active drugs. The evaporation of part of ethanol allows the contact between lipids (Transported by bubbles) and water, and favors the formation of liposomes.[40]

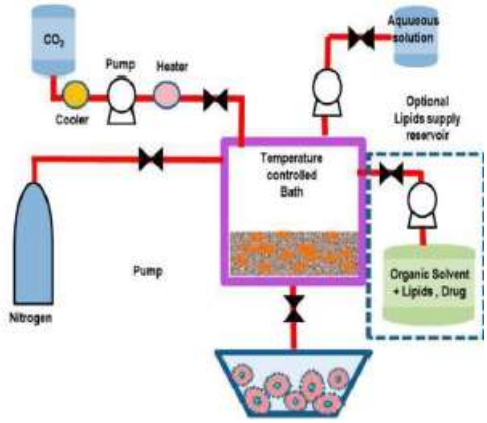
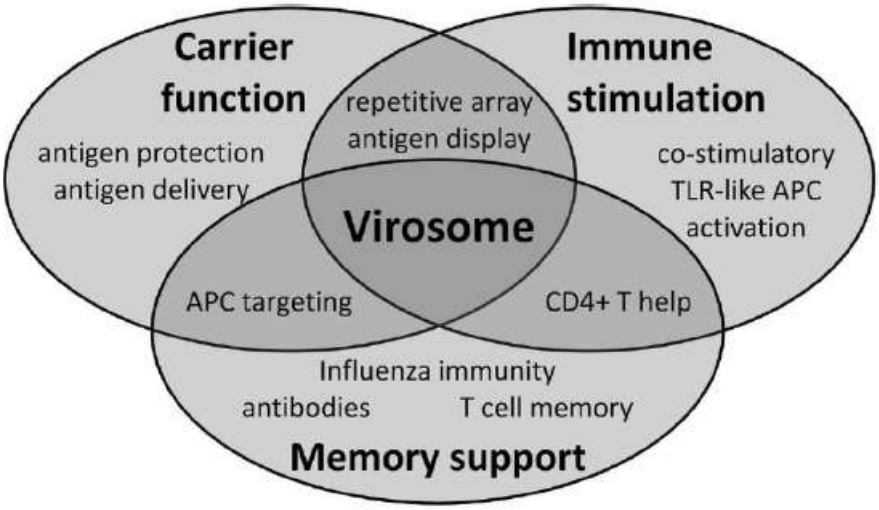


Fig. 22: Prepration flowchart of DELO.

Virosomes on liposomal drug delivery

Introduction of virosomers: Virosomes sound quite interesting and promising in the field of drug delivery. They are actually viral envelopes that have been reconstituted and madesafe for therapeutic use by removing the viral genetic material. They have the ability to encapsulate drugs within their lipid bilayers, or attach them to their surfaces. One of the biggest advantages of virosomes is their ability to target specific cells or tissues by modifying their surface proteins, which enhances drug efficacy while minimizing side effects on healthycells. Additionally, the lipid bilayer of virosomes protects encapsulated drugs from degradation, thus improving their stability and bioavailability. Virosomes also possess intrinsic adjuvant properties, which can stimulate the immune system. They can be used in vaccine development, where they act as carriers for antigens, triggering a robust immune response. Another advantage of virosomes is their versatility, as they can encapsulate various types of drugs, including small molecules, peptides, and nucleic acids. Finally, virosomes are generally well-tolerated by the body, reducing the risk of adverse reactions. irosomes are indeed fascinating structures that have shown great promise in drug delivery. They are essentially viral envelopes that have been reconstructed without the viral genetic material,

making them safe to use therapeutically. What makes virosomes unique is their ability to encapsulate drugs within their lipid bilayers or attach them to their surfaces, offering several advantages. One of the most significant advantages of virosomes is their targeted delivery system. They can be engineered to target specific cells or tissues by modifying their surface proteins. This targeted delivery enhances drug efficacy while minimizing side effects on healthy cells. Additionally, the lipid bilayer of virosomes protects encapsulated drugs from degradation, improving their stability and bioavailability. Virosomes also possess intrinsic adjuvant properties, which can stimulate the immune system. This makes them ideal for vaccine development, where virosomes act as carriers for antigens, triggering a robust immune response. Their versatility is another advantage, as virosomes can encapsulate various types of drugs, including small molecules, peptides, and nucleic acids, making them suitable for delivering a wide range of therapeutic agents. Lastly, virosomes are generally well-tolerated by the body, reducing the risk of adverse reactions. Their biocompatibility, combined with their targeted delivery and enhanced drug stability, make virosomes a promising drug delivery system with immense potential in the field of medicine.



A virosome is a drug or vaccine delivery mechanism consisting of unilamellar phospholipid membrane (Either a mono- or bi-layer) vesicle incorporating virus derived proteins to allow the virosomes to fuse with target cells. Viruses are infectious agents that can replicate in their host organism, however virosomes do not replicate. The properties that virosomes share with viruses are based on their structure; virosomes are essentially safely modified viral envelopes that contain the phospholipid membrane and surface glycoproteins. As a drug or vaccine delivery mechanism they are biologically compatible with many host organisms and are also

biodegradable. The use of reconstituted virally derived proteins in the formation of the virosome allows for the utilization of what would otherwise be the immunogenic properties of a live-attenuated virus, but is instead a safely killed virus.[1] A safely killed virus can serve as a promising vector because it won't cause infection and the viral structure allows the virosome to recognize specific components of its targetcells.

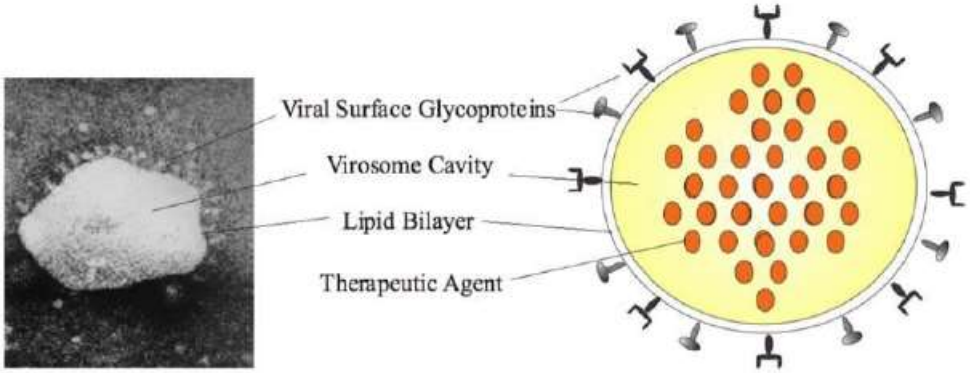


Fig. 23: Basic structure of liposomes.

Virosomes are vehicles that have a spherical shape with a phospholipid mono/bilayer membrane. Inside of the virosome, there is a central cavity that holds the therapeutic molecules such as nucleicacids, proteins, and drugs. On the surface of the virosome, there can be different types of glycoproteins. Glycoproteins are a type of protein that have an oligosaccharide chain bonded to amino acid chains. The different types of glycoproteins on the surface of the virosome increases the specificity of the target cells because the surface glycoproteins help with recognition as well as the attachments of the virosomes to their target cells. In the case of the influenza virosome, the glycoproteins are antigen, haemagglutinin, and neuraminidase. Antigens are molecules that triggers an immune response when targeted by a specific antibody that corresponds to the shape of the antigen.[3] Haemagglutinin is a viral glycoprotein that causes red blood cell agglutination. Neuraminidase are enzymes that break glycosidic linkages.[5] The size and surface molecules presented on of the virosome can be modified so that it can target different types of cells.

Uses of virosomes

1. **Targeted delivery:** Virosomes can be engineered to target specific cells or tissues by modifying their surface proteins. This enhances drug efficacy while minimizing side effects on healthy cells.
2. **Enhanced drug stability:** The lipid bilayer of virosomes protects encapsulated drugs

fromdegradation, thus improving their stability and bioavailability.

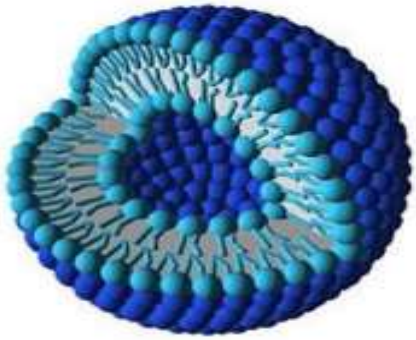
3. **Immunogenicity:** Virosomes possess intrinsic adjuvant properties, which can stimulate the immune system. This property is exploited in vaccine development, where virosomes act as carriers for antigens, triggering a robust immune response.
4. **Versatility:** Virosomes can encapsulate various types of drugs, including small molecules, peptides, and nucleic acids. This versatility makes them suitable for delivering a wide range of therapeutic agents.
5. **Biocompatibility:** Virosomes are generally well-tolerated by the body, reducing the riskof adverse reactions.

Significant of virosomes in liposomal drug delivery system

Virosomal liposomal drug delivery systems are created by integrating virosomes with liposomes. A crucial step in this process is the fusion of these two entities. Several methods have been developed to achieve this fusion efficiently. Below are some commonly used methods for virosome-liposome fusion:

- ✓ The Membrane Extrusion Method is a process that involves the extrusion of both virosomes and liposomes through porous membranes under controlled conditions. To begin, virosomes and liposomes are mixed together and subjected to extrusion through polycarbonate membranes with defined pore sizes. The mechanical force generated during extrusion promotes fusion between virosomes and liposomes, leading to the formation of virosomal liposomes. This method is particularly advantageous as it allows for the control of vesicle size and uniformity, which is crucial for drug delivery application.

- ✓ pH-Induced Fusion is a process that involves taking advantage of the pH-sensitive properties of virosomes and liposomes to cause fusion. By adjusting the pH levels of the surrounding medium, it becomes possible to induce fusion between virosomes and liposomes. For instance, under acidic pH conditions, fusion can occur by promoting conformational changes in the viral fusion proteins found on the virosomal membrane. This method is simple and versatile, as it doesn't require complex equipment or procedures.



- ✓ Chemical fusion methods involve the use of fusogenic agents or compounds to facilitate fusion between virosomes and liposomes. Fusogenic agents such as polyethylene glycol (PEG) or fusogenic peptides can be incorporated into virosomes or liposomes. These agents promote membrane fusion between virosomes and liposomes when exposed to specific conditions such as changes in temperature or pH. Chemical fusion methods provide precise control over fusion parameters and can be customized to achieve optimal fusion efficiency.

- ✓ Electrofusion is a process that involves the use of an electric field to fuse virosomes and liposomes together. This process is carried out by immobilizing virosomes and liposomes on electrode surfaces and applying an electric field to promote the fusion of their membranes. The electric field destabilizes the lipid bilayers of both virosomes and liposomes, which results in the formation of virosomal liposomes. Electrofusion is known for its high fusion efficiency and ability to produce virosomal liposomes with controlled size and composition.

- ✓ Lipid exchange is a technique that involves the transfer of lipids between virosomes and liposomes, ultimately resulting in the fusion of their membranes. This process is facilitated by the addition of lipid exchange-promoting agents such as detergents or lipid exchange proteins. By mixing virosomes and liposomes together in the presence of these agents, the lipids undergo exchange, leading to the formation of virosomal liposomes. This method is considered simple and efficient, requiring minimal equipment and manipulation.

- ✓ Sonication is a process that involves the application of ultrasonic waves to induce fusion between virosomes and liposomes. The ultrasonic waves generate mechanical energy that promotes membrane fusion between virosomes and liposomes. This disruption of lipid

bilayers facilitates the formation of virosomal liposomes. Sonication is an efficient and rapid method for virosome-liposome fusion. However, it is important to optimize sonication parameters to prevent excessive vesicle disruption.

There are various methods available to combine virosomes and liposomes to create fusion vesicles, each with its own set of advantages and limitations. The selection of a particular fusion method depends on factors such as the desired size of the vesicles, their composition, and the intended use. It is crucial to optimize the fusion parametersto ensure the efficient and consistent formation of virosomal liposomes that can be used for drug delivery application.

Composition and Structure of virosomes over liposomes

Virosomes and liposomes are both lipid-based vesicles used in drug delivery, but they differ in their composition, structure, and origin. Understanding these differences is crucial for harnessing their unique properties in drug delivery systems.

Lipids: Virosomes are similar to liposomes as they both contain a lipid bilayer composed of phospholipids and cholesterol. However, the lipid composition of virosomes may vary depending on the source virus and the method of virosome preparation. Commonly found phospholipids in virosomes include phosphatidylcholine and phosphatidylethanolamine.

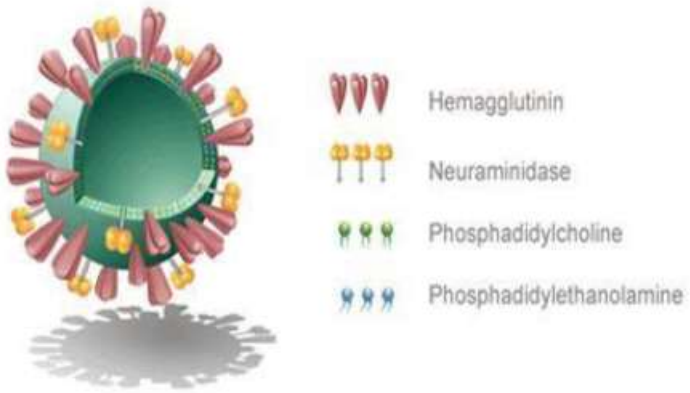
Viral proteins: Virosomes are special structures that keep important viral membrane proteins such as fusion proteins and antigens. These proteins play a crucial role in the ability of virosomes to fuse with target cells and stimulate the immune system. This makes virosomes a great option for using in vaccine delivery and immunotherapy treatments.

Virosome structure

Virosomes are consist of spherical or unilamellar phospholipidbilayer vesicle having meandiameter in the range of 120-180nm. Influenza virus is most commonly used for virosome production and genetic material of the sourcevirus. Virosomesarenotcap able to replicate other than purefusion active vesicles are presents.

The Virosomemainly constituentsof Immune stimulating Regenerate Influenza Virosomes (IRIVs) consist of naturally occurring phosphatidylcholine (PC) and phospholipids (PL). PC formsaround 70% of the virosomal structure. The left behind 30%of membrane components has the envelopephospholipids originating from the influenza virus to facilitate provide haemagglutinin(HA) and neuraminidase (NA) glycoproteins.Virosomes canbe optimized

usedfor maximal inclusion of thedrug or for the greatest physiological effect bymodifying the content or else type of membranelipids used. It is even possible to generatecarriers for antisense-oligonucleotides orothersome genetic molecules depending onwhether positively or negatively loadedphospholipids are included into themembrane. Various ligandslike peptides, cytokines, and monoclonalantibodies (MAbs) can be incorporated intothe virosome. It also displayed on the virosomalsurface. Tumor-specific monoclonalantibody fragments (Fab) might be linked tovirosomes to direct the carrier to selectedtumor cells.



Structure similarity with liposomes

Morphology and lipid bilayer composition, they differ in origin, membrane protein content, and functional propertie.

Spherical vesicular morphology: Virosomes and liposomes have similarities in their morphology and lipid bilayer composition; however, they differ in origin, membrane protein content, and functional properties.

Lipid bi-layer composition: Both virosomes and liposomes are composed of a lipid bilayer containing amphiphilic phospholipids and cholesterol, which provide stability and biocompatibility for the encapsulation of hydrophilic and hydrophobic drugs.

Encapsulation wuth Drugs and Biomolecule: Virosomes and liposomes are efficient carriers that can encapsulate drugs, nucleic acids, proteins, and other biomolecules in their aqueous core or lipid bilayer. This protective barrier shields the payload from degradation and enablescontrolled release at the intended location.

Biocompatibility and Biodegradability: Virosomes and liposomes are biocompatible and

biodegradable, making them suitable for biomedical applications. They are well-tolerated by the body and do not elicit significant immune responses or toxicity.

Preparation of virosome

1. **Selection of virus**

Virosome are recons titute dviralenvelop which have consequent from different virus. Influenza virus envelope is most of ten used to produce virosome but virosome can also be made from Sendai virus, sindbis, Epstein-burrvirus, friendmurine leukemia virus, herpes simplex virus.[2]

2. **Selection ofantigen**

Antigen is preferred as per our necessities.Antigenswhich are used like abacterium parasite, carcinogenic cell, orwholecell is used as antigens. Cell components includeRNA, DNA, or plasmid could also be used as antigen.[3]

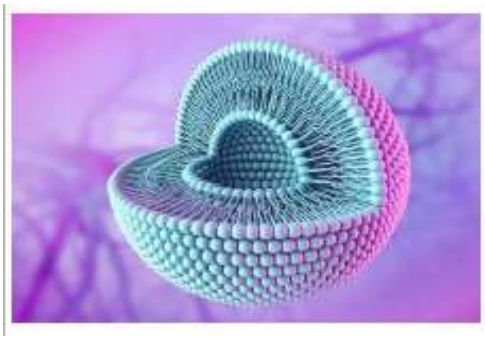
3. **Reconstituted of virosome**

Virosome solubilized with detergent such as (octaglucoside, nonidert p-40). Due to solubilization with detergent genetic material and internal viral protein will sediment afterward detergent is removed by different method like hydrophobic resins and dialysis from supernatant. By uses ultracentrifugation development viral matrix protein and nuclei capsid is removed. Antigen which has coupled to lipid anchor is mixed with surfactant or polymer solution. This solution is process among virosome mover antigen bound virosome is achieved.

Advantages of virosomal drug delivery

- Enable medication conveyance into the cytoplasm of target cell
- Virosomes are biodegradable.
- Protects drugs against corruption.
- Biocompatible, and non-lethal
- No ailment transmission hazard
- No auto immunogenity
- Broadly material with terrifically imperative medications (Anticancer drugs, Proteins, peptides, Nucleic acids, Anti-infection agents, Fungicides)
- Promotes combination movement in the endolysosomal pathway
- Target-specific delivery of antigen sand amplification of the immune response.
- Extended uptake, distribution and elimination of drug in body.

- Up scaling according to standard procedure.
- Virosome allow patient specific modular vaccine regimen.
- Can be regulated by injection or nasally.
- Release of active substance in cytosol of selected cell. The anti genis partially protect ed from extra cellular degradation and theres ulting depot effect greatly facilitates immune potentiation.
- Target-specificdeliveryofantigensandamplificationoftheimmuneresponse.
- Virosome allow tolerant specific modular vaccine regimen.



Characterization of virosomesprotein detection

Virosome preparation could be usually consequence in the comparatively consistent proteinto lipid ratios. For the confirm the presence of HA protein in the virosomes the agent Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) are used.

Structure and Size

For the Negative stain electron microscopy should be normally be used to determine the ultrastructure and size of virosomes. The Prepared staining solutions could be rather be of neutral pH for avoid acid induced conformation alteration of HA.

Fusion activity

Regularly virosomes show pH dependent film combination movement like local flu infection. Virosomal combination with organic or counterfeit target films can be surveyed in vitro with an excimer measure utilizing pyrene-named lipids, where the decline of surface thickness of the pyrene-phosphatidyl choline-name on combination with an unlabeled layer compares to a decrease of excimer fluorescence. Combination action additionally can be in a roundabout way checked by deciding hemolytic action, which relates nearly to combination action and displays pH reliance indistinguishable with that of combination.

Evaluation of virosomes

A) **Surface Morphology and Vesicle shape: -** Transmission electron microscopy, Freeze breakelectron microscopy.

B) **Size Dispersion and Vesicle size:-** Dynamic light scrambling, Transmission electron microscopy, Zetasizer, Photon connection spectroscopy, Laser light diffusing, Gel saturationand gel avoidance.

C) **Surface charge: -** Free stream electrophoresis.

D) **Surface pH and electrical surface potential: -** Zeta potential estimations and pH touchy tests.

E) **Lamellarity: -** Small edge x-beam dissipating, Freeze break electron microscopy, 13p-NMR.

F) **Phase conduct:** Freeze crack electron microscopy, Differential checking colorimetry.

G) **Percent of free medication:** Mini section centrifugation, Gel avoidance and Ion trade chromatography, Protamine accumulation, Radiolabelling.

H) **Drug discharge:** Diffusion cell/dialysis.

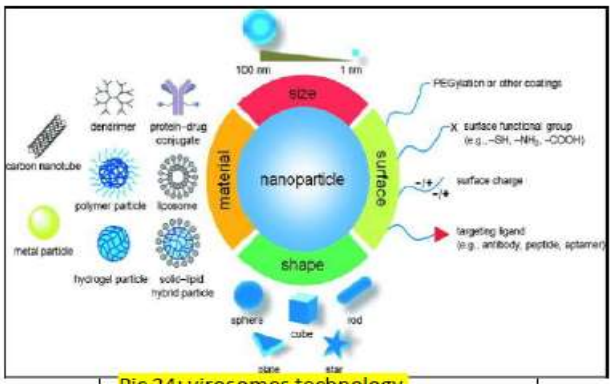
I) **Pyrogenicity:** Rabbit fever reaction test or Limulus ambeocyte lysate (LAL) test.

J) **Animal poisonous quality:** Observing survival rates, histology and pathology.

K) **Chemical examination of surface:** Static auxiliary particle mass spectrometry.

Virosomal technology

Viruses are commit intracellular parasites as they are fundamentally subordinate upon particular host cellsfor their survival. This guideline leadsto the improvement of a medication conveyance framework that copies the viral example of cell disease. Virosomes are made out of aphospholipid bilayer with the viral surface glycoproteins distending from the surface of these vesicles.



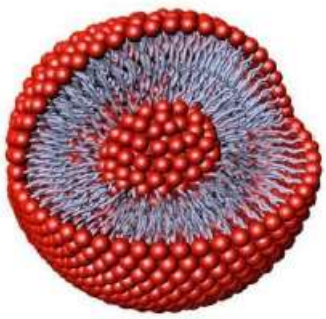
Pic 24: Virosomes technology.

The synthesis of the vesicular layer empowers the virosomes to be biocompatible and biodegradable. They are productively consumed and circulated to the objective site without being modified by the physiological procedures of the body. Additionally, the plan and structure of virosomes is to such an extentthat medication particles of various nature can be consolidated in them. The lipid bilayer can effortlessly incorporate the hydrophobic medications in it. Hydrophilic medications, on the other hand, turn into a piece of the focal lacunae. Virosomes can be coupled to an immune response to guarantee the focused on conveyance of a helpful operator to upgrade the tissue specificity. These antibodies tie to the particular receptors of cells supporting the conveyanceof medication atoms to these objectives. This property can, particularly, be used for conveying the medication particles with limit security profiles. Disease chemotherapeutic operators, for example, can be conveyed particularly to the tumors by marking the virosomes with antibodies. Virosomes have appeared to viably transport macromolecules including drugs, nucleic acids and proteins to different cell sorts including hepatocytes, erythrocytes, safe cells and gliomacells. Variousvirosome based items have been affirmed by the United States Food and Drug Administration (FDA) for human utilize. The surface glycoproteins of Influenza infection, hepatitis infections and vesicular stomatitis infection have been effectively consolidated in various antibody and medication conveyance system. Virosomes containing malignancy chemotherapeutic specialists, antimalarial, antibacterial and antifungal operators have appeared effective discharge profiles in vitro and in vivo. In view of the same standard, bacterial apparitions have been created. These vesicles contain the external shell or the encompass protein of different gram negative microscopic organisms. These bacterial apparitions emulate a comparative design as is seen in the event of a characteristic disease. The virosome based sedate conveyance is, in any case, quick, sheltered and viable rather than other related system.

Virosomes as immuno potentiating agents

Virosomes are the agents that can serve the function of delivering antigens and drugs to specific cell types. The chief property exploited throughthe virosome design is the interaction amongst the antigenic proteins of the virus with the cellular receptors. Moreover, the identification, uptake and representation of the antigen incorporated in the virosomeby the relevant antigen presenting cells helps in stimulating the immunesystem. As a result efficient regulatory and effector immune responses aregenerated. They initiate both cell mediated andhumor alarms of immune system. Additionally, virosomes induce bothcytotoxic and

helper T-cell responses.[27-29] Virosomes cannot only serve as a means to transfer the immunogen to the body but can also actas adjuvants for directing the immune response to the particular antigen. They, being of particulate nature, can easily attract the dendritic cells and other antigen presenting cells for attaining immunological benefits. The composition of thevirosome ensures that the antigen, whether intercalated into the lipid bilayer, conjugated to the surface proteins or present in the central cavity, is delivered continuously in a sustained manner to the immunesystem. This delay in the release of the antigen can act as a tool for focusing the immune response to the particular antigen in order to gain a depot-like effect. Furthermore, the combined delivery of the antigen and the adjuvant can help in the attainment of an exaggerated immune protection against various diseases. Recent studies on murine models have exhibited up to four-fold improved humoral response in case of virosome based product in comparison to that observed on the delivery of nascent antigen.



Virosomes agents oftargeted drug delivery

One of the imperative essentials of a medication conveyance framework is to transport a remedial specialist successfully to the objective site in an auspicious way. Keeping in mind the end goal to help the focused on sedate conveyance, drugs should be either changed or bundled in such a way, to the point that restoratively successful amounts of medication atoms achieve the site of activity. Change might include the modification of physical as well as synthetic parameters of the medication bringing about the generation of new synthetic elements, blending with other substance constituents to alter their in vivo discharge profilesor, on the other hand change of physical structures of the medication particles. Virosomes can bundle medications of an assortment of nature in themselves.[33-34] They can fill in asfantastic intends to convey hydrophilic and hydrophobic medication atoms to a particular kind of tissue. The water-cherishing or hydrophilic medications are epitomized in the focal compartment amid the virosome generation prepare. The lipophilic drugs, then again, can'tbe epitomized in this way and are, along these lines, installed in the lipid bilayer. The moderate

breaking down what's more, disintegration of the virosomes inside the cell can fill in as a methods of conveying these medication particles to the expected site of activity. The exemplification of different types of hereditary material in the virosome, to be utilized for prophylactic or helpful purposes, has been accomplished in various examinations. The lipid bilayer of the virosome makes a difference in the assurance of these remedial operators from different nucleic corrosive corrupting proteins including DNAases and RNAases. The viral glycoproteins subsequent to perceiving the particular cell sorts help in the combination of the films. The hereditary material once conveyed can, at that point, be used by the cell hardware for the creation of the encoded qualities.

Virosome-Cell

Interaction

The main preferred standpoint of the virosome innovation is their ability to mimic an in vivo contamination express that can be useful in drawing in the resistant players and the arrangement of macromolecules to the separate site of activity. Virosomes perceive and tie to the same receptors that are used if there should be an occurrence of a characteristicviral disease. Sialic corrosive receptors, for example, are used by the flu virosomes. After the cell receptor acknowledgment by the infection, combination of viral and endosomal layer is watched. If there should arise an occurrence of flu virosomes, for instance, the hemagglutinin (HA) viral protein uses its dipartite get together for a similar reason. Furthermore, the neuraminidase (NA) is likewise incorporated into the virosome get together as it can upgrade the immunogenicity and focusing of the virosome to a specific tissue. The virosome-receptor connection has been explored for the treatment of various sicknesses including parasitic illnesses, viral maladies, neurological clutters and numerous other metabolic issue. In every one of the cases, the principle point is the arrangement of a nano-sized protein, nucleic corrosive or a medication particle to the expected site of activity. Peptides and proteins have been effectively conjugated with the virosome-surface glycoproteins. Immunizations have been produced against the Respiratory Syncytial Virus (RSV) utilizing the flu virosomes by intertwining the monitored proteins of the Hepatitis C infection surface proteins with the virosomal proteins positive enlistment of cytotoxic White blood cell insusceptible reaction. Correspondingly, epitopic locales of B-cell have been conceived utilizing flu virosomes particularly against intestinal sickness. Moreover, numerous pathogens have been focused on utilizing the virosome framework. This system, along these lines, helps in maintaining a strategic distance from rehashed and different dosing for vaccination purposes.

Pharmacokinetics of virosomes

Pharmacokinetics data can be utilized to translate the distinctions in the pharmacological impact of liposomal-entangled medication and free medication, consequently can be abused for measurements planning. Pharmacokinetics manages time course of retention, dissemination and debasement of the virosomal transporters in vivo. The pharmacokinetics of virosomes requires the information of conceivable available locales after intravenous organization as this is the most acknowledged course for different virosomal details misused for clinical therapeutics with the exception of topical plans. Virosomes modifies both the tissue dissemination and the rate of leeway of a medication as they are influenced by the pharmacokinetics parameters. Under ideal conditions the medication has been conveyed inside the virosomal fluid stage amid flow and it spills at adequate rate to wind up noticeably bioavailable on landing in tissue or other particular destinations. Bioavailability in the event of virosomal transporters can be characterized as the measure of free medication that can get away from the bounds of the bearer and in this manner end up plainly accessible for redistribution to neighboring tissue.

Mechanism of action of virosomes

Virosomes act both as a medication conveyance transporter and furthermore as an adjuvant with different capacities amid the enlistment of a safe reaction to body's. The transporter work contains the beneficial outcomes of implanting the antigen into a higher structure, the virosome molecule. The adjuvant capacity identifies with immune stimulating properties of the virosomes and their segments on the resistant framework. Above all, virosomes prevail with regards to invigorating particular invulnerability without causing nonspecific aggravation.

Current Research and Challenges

- **Personalized medicine:** Customizing virosomes for individual treatments.
- **Multifunctional virosomes:** Combining drug delivery with diagnostic imaging.
- **Production and Scalability:** Ensuring consistent and high-quality manufacturing.
- **Immune response:** Managing potential immune reactions against virosomecomponents.
- **Regulatory approval:** Meeting stringent safety and efficacy standards.

CONCLUSION

Virosomes are innovative tools for targeted drug delivery, leveraging the structure of viral envelopes to deliver therapeutic agents specifically to target cells. They offer the advantages

of high specificity, safety, and versatility, making them suitable for applications in cancer therapy, vaccines, gene therapy, and infectious diseases. While challenges such as production scalability and immune responses exist, ongoing research aims to address these issues and enhance the efficacy and applicability of virosomes in modern medicine.

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