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REVIEW ON LIPOSOMES: A POTENTIAL DRUG DELIVERY TECHNOLOGY

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Abstract:-

Liposomes are thought to be diverse and promising drug delivery systems. When it comes to site-targeting, regulated or sustained release, shielding medications from deterioration and elimination, better therapeutic outcomes, and less harmful side effects, liposomes outperform conventional drug delivery methods. Because of these benefits, during the past few decades, a number of liposomal medicinal items have beenapproved and successfully used in clinics. This overview covers the liposomal drug products that have been approved by the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) in the United States. The marketed liposomal products' lipid excipient, manufacturing processes, nanosizing technique, and drug formulation are all based on the FDA's published approval package and the European Public Assessment Report (EPAR) in the EMA, as well as critical chemistry information and sophisticated pharmaceutical technologies. Critical quality attributions (CQAs) of items and loading techniques are presented. Furthermore, a summary of the most recent regulatory advice and anticipated developments pertaining to liposomal products is provided. This information can be applied to the study and development of liposomal drug candidates in a number of settings, such as pilot plants, commercial manufacturing facilities, and lab benches.

Keywords: Drug loading, lipid excipient, drug delivery, and liposomes.

Introduction

Liposomes are drug-based, self-assembling phospholipid-based vesicles that surround a core aqueous compartment in the shape of a concentric series of several bilayers (multilamellar) or a bilayer (uni-lamellar) [1]. The phospholipid bilayer of liposomes is 4-5 nm thick, and their sizes range from 30 nm to the micrometer scale [2]. At Babraham Cambridge, British scientist Alec Bangham and associates established the field of liposomology inthe middle of the 1960s [3], and the structure of liposomes was originally reported in 1964 [4]. Since then, a great deal of research has been done on liposomes as delivery systems for protein, nucleic acid, small molecules, and imaging agents [5,6,7,8,9]. To increase patient compliance and treatment efficacy, various delivery routes, including parenteral, pulmonary, oral, transdermal, ocular,

and nasal routes, have been established [10,11,12,13, 14]. Furthermore, liposomes have been used extensively in the food and cosmetics industries[15, 16].

Liposomes, as drug vesicles, are exceptional in that they shield the enclosed materials from physiological degradation [17], prolong the drug's half-life [18], regulate the release of drug molecules [19], and have good biocompatibility and safety. Additionally, liposomes can use passive and/or active delivery methods to precisely deliver their payload to the sick region. active targeting, enhancing the therapeutic advantages, raising the maximum tolerable dose, and reducing the systemic side effect [19,20]

Highly porosity capillaries (ranging from 100 nm to 2 µm, depending on the size and type of tumor tissue) are present in aberrant tissues, such as solid tumors and inflammatory sites, in contrast to normal tissue, which has tight intracellular junctions (2–6 nm) between endothelial cells [21, 22]. The enhanced permeability and retention (EPR) effect is the ability of liposomes to passively concentrate and retain at the aberrant tissues after crossing the discontinuous neovasculature. Active targeting makes use of particular interactions between the liposome and tumorcell surface ligands and receptors, respectively.

- **Liposome Structures and Principal Elements**

Depending on the compartment structure and lamellarity, liposomes can be categorized as unilamellar vesicles (ULVs), oligolamellar vesicles (OLVs), multilamellar vesicles (MLVs), or multivesicular liposomes (MVLs) (Figure 1) [23]. Although OLVs and MLVs have a structure similar tothat of anonion, they also have two to five or more concentric lipid bilayers.

Unlike MLVs, MVLs have a structure resembling a honeycomb and contain hundreds of non-concentric aqueous chambers surrounded by a single bilayer lipid membrane [22]. ULVs can be further classified as small unilamellar vesicles (SUVs, 30-100 nm), large unilamellar vesicles (LUVs, >100 nm), and giant unilamellar vesicles (GUVs, >1000 nm) [25] based on the size of the particles. A range of sizes for ULVs was reported, including SUVs that are smaller than 200 nm and LUVs. between 200 and 500 nm in size [26].

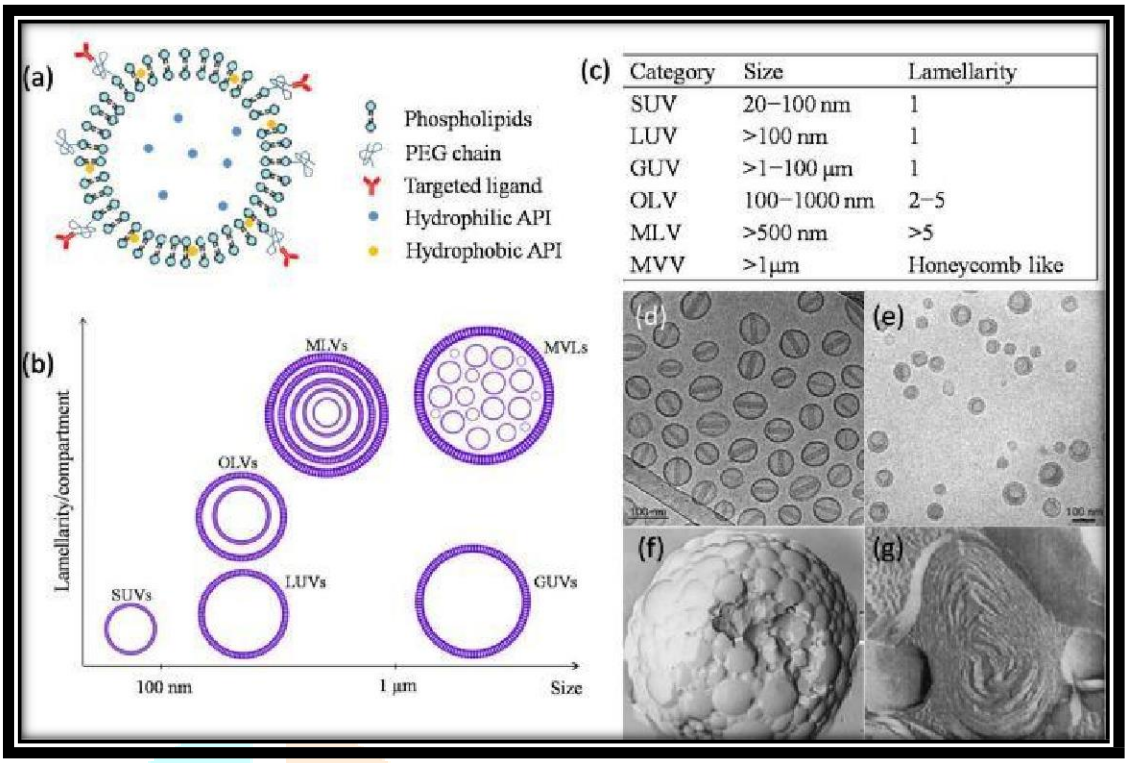

Figure no.1 Liposomal Drug Delivery System Structures and Categories

Liposomal drug delivery system classifications and structures. (a) A structural diagram showing the composition of liposomes. The typical sizeof a phospholipid bilayer is 4.5 nm, which is significantly smaller than the inner aqueous core; (b) Liposomal vesicles are classified based on their lamellarity/compartment and particle size; (c) Different types of liposomes' size and lamellarity; (d, e) Cryo-transmission electron microscopy of Doxil [26] and Vyxeos [27]; (f, g) Electron micrographs of DepoFoamTM particles (e.g., DepoCyt) and MLVs (e.g., Mepact) [28].

The primary components of liposomes are cholesterol (Chol), sphingomyelin (SM), and glycerolphospholipid (GP). are the fundamental elements found in the products that are sold. Glycerol, which connects two hydrophobic fatty acid chains and a hydrophilic polar head group, ispresent in GP [29]. Figure 2a describes the various polar heads and fatty acid types. Different head groups produce liposomes with neutral (PC and PE) or negative (PA, PS, PG, and cardiolipin) charges at physiological pH [30].

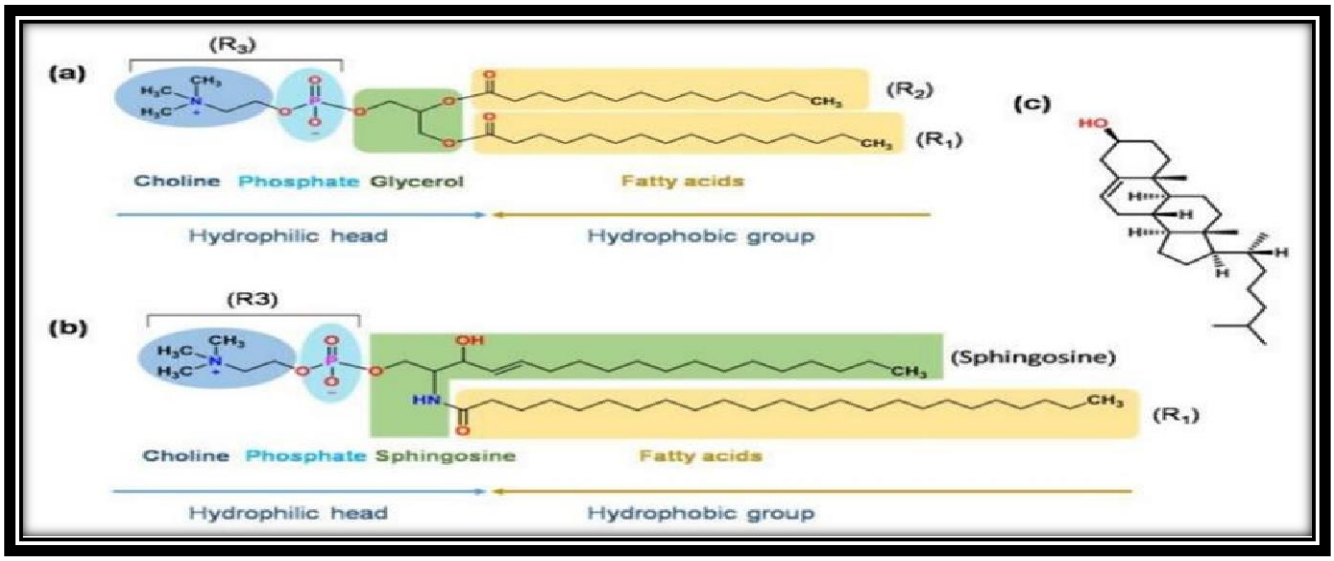

Figure 2:structural makeup of cholesterol, sphingomyelin, and glycerol phospholipid

- **The Production Procedure**

Numerous techniques for preparing liposomes have been developed. Figure 3 [79] summarizes the possible manufacturing processes of the liposomal products that are marketed, based on relevant publications and patents. The double emulsion method, ethanol injection, and thin-film hydration are among the frequently employed manufacturing techniques. First, depending on the method chosen, the processes involve

(1) making MLVs or ULVs; (2) size reduction if required; (3) making the drug solution(s) and drug loading; in the case of passive drug loading, this step is combined with step 1; (4) buffer exchange and concentration if necessary; (5) sterile filtration or aseptic processing; and (6) lyophilization, if necessary, and packaging.

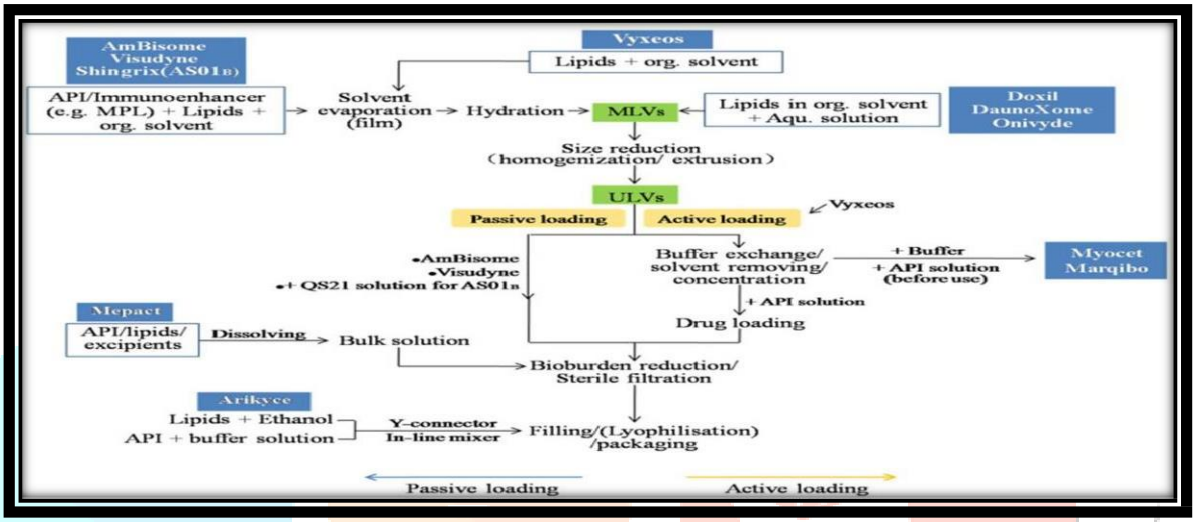

Figure No. 03: The possible production methods for the liposomal products that are sold

- **Liposome Arrangement**

- **Film-Hydration Technique**

One conventional method that works well for loading lipophilic drugs is thin-film hydration. As the flask rotates under vacuum, the lipid– solvent solution evaporates, forming a thin film. By hydrating the lipid film with the aqueous solution, MLV suspension can be produced. SUVscan be created by further reducing the particle size, and the drug can be added either passively or actively during or after the liposome forms.

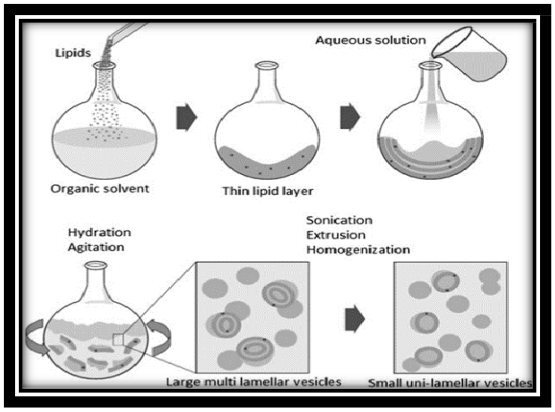

Figure No. 04: Method of thin-film hydration

- **Method of Double Emulsification [32]**

In order to create a primary w1/o emulsion, an active ingredient is first dissolved in an aqueous phase (w1) and then emulsified in anorganic polymer solvent.

To create a w1o/w2 double emulsion, this primary emulsion is further combined with an emulsifier that contains an aqueous solution(w2).

When the solvent is removed, microspheres remain in the aqueous continuous phase, where they can be gathered using centrifugationorfiltering.

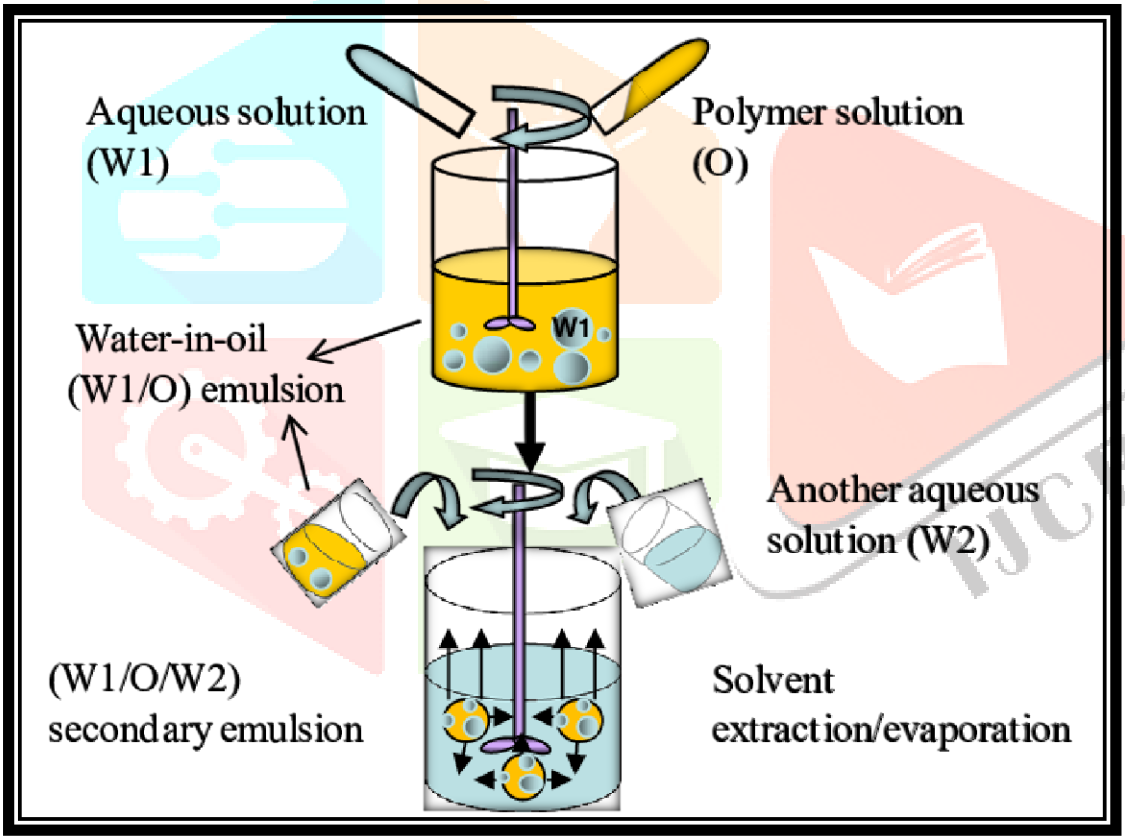

Figure No. 5: Double Emulsification Technique

- **Methods of Solvent Injection**

To create liposomes, lipids are added to an organic phase (ethanol, ether, or chloroform) and then injected into an aqueous phase using thissolvent injection method [44]. Depending on the solvent used, this method again split into two sub-methods.

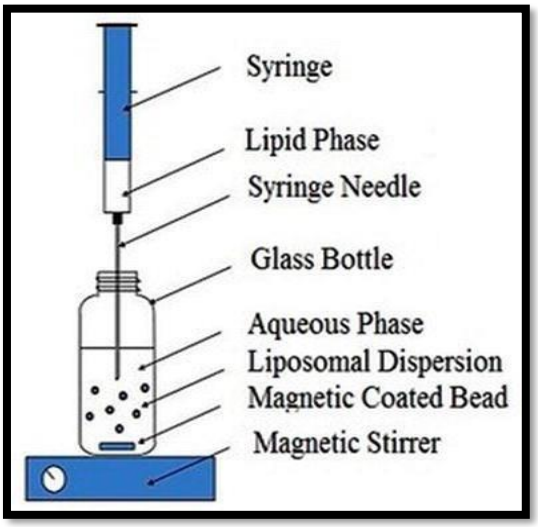

Figure No. 06: Methods of Solvent Injection

- **Size-Reduction Strategies:-**

The performance and safety of liposomes are significantly dependent on their size and size distribution. There are numerous techniques for reducing the size of liposomes, including (ultra)sonication using a bath or probe, homogenization, French press [43], extrusion, and combination techniques like freeze-thaw sonication, freeze-thaw extrusion, and a high-pressure homogenization–extrusion method. Extrusion and high-pressure homogenization (HPH) are the methods most frequently employed in the production of pharmaceuticals among these.

Drug-Loading Techniques o Passive Drug-Loading Strategy Encapsulating the drug agent during liposome preparation is the passive drug- loading method. The medication can be encapsulated in the inner aqueous space or embedded in the liposome bilayer through interactions that are covalent, ionic, electrostatic, non-covalent, or steric.

- **Drug-Loading Techniques:-**

O Passive Drug-Loading Strategy Encapsulating the drug agent during liposome preparation is the passive drug-loading method. Drug molecules can interact with lipids in a covalent, ionic, electrostatic, non-covalent, or steric manner to encapsulate the drug inside the inneraqueous space or embed it within the liposome bilayer. This method's primary drawback is its poor encapsulating efficiency, which necessitates an extra step of free drug removal. Using the passive drug loading method, marketed liposomal products like AmBisome, Visudyne, Arikayce, DepoCyte, DepoDur, and Expel have been identified through patents and publications.

O The active drug-loading method The drug agent is loaded using the active drug loading technique, also known as remote drug loading, after empty liposomes are created. The membrane The medication is encouraged to diffuse across the membrane and enter the inner core ofliposomes by a gradient of pH or ion concentration. It takes five to thirty minutes to entrap drugs, and a high loading efficiency (above 90%) can be attained.

- **Drug-Lipid Interaction through Covalent Bonding**:

Another effective method of loading the drug into liposomes is to covalently link the drug molecules to lipids using a linker; one such product is Mepact. The component of most Gram-positive bacteria's cell walls, muramyl dipeptide (MDP), has the ability to strengthen immune responses. Because MDP is a low-molecular-weight, water-soluble molecule, the MDP liposome exhibits issues such as low entrapment efficiency and drug leakage during storage. MTP-PE (muramyl tripeptide-phosphatidyl ethanolamine) was created by attaching MDP to PE via a peptide spacer in order to increase the lipid solubility of MDP. The amphipathic molecules of MTP-PE intercalated into the liposome membrane bilayers when the lyophilized product (POPC, OOPS, and MTP-PE) was reconstitution with saline solution. MTP-PE was present in no free MTP- PE was discovered in the liposomes [43].

- **Combination Approach**

Vyxeos is the first approved liposome loaded with two different drugs (cytarabine and daunorubicin) in the same vesicle. It is loaded both passively and actively. In summary, hydrating the lipid foams with a solution of Cu(gulconate)2, triethanolamine (TEA), pH 7.4, and cytarabine results in the passive encapsulation of cytarabine into liposomes. Following sizing reduction and buffer exchange to eliminate the unencapsulated drug and Cu(gulconate)2/TEA, the cytarabine-loaded liposomes are incubated in a daunorubicin buffer solution at neutral pH. Cu(gulconate)2/TEA-based loading is used to actively accumulate daunorubicin inside liposomes. While the neutral form of TEA permeates towards the extraliposomal medium, daunorubicin diffuses through the lipid bilayer into the intraliposome, establishing a stoichiometric and kinetic connection between daunorubicin and the TEA efflux. Cu(gulconate)2/TEA is essential for the interaction between the two medications,allowing the retention of each drug inside liposomes and controlling the release of the drug from them [36].

- **Evaluation parameters:**

Liposomes produced by different methods have varying physicochemical characteristics, which leads to differences in their in vitro (sterilization and shelf life) and in vivo (disposition) performances. Rapid, precise and reproducible quality control tests are required for characterizing the liposomes after their formulation and upon storage for a predictable in vitro and in vivo behavior of the liposomal drugproduct.

- **Size and size distribution:**

When liposomes are intended for inhalation or parenteral administration, the size distribution is of primary consideration, since it influences the in vivo fate of liposomes along with the encapsulated drug molecules. Various techniques of determing the size of the vesicles include microscopy (optical microscopy, negative stain transmission electron microscopy, cryo-transmission electron microscopy, freeze fracture electron microscopy and scanning electron microscopy, diffraction and scattering techniques (laser light scattering and photon correlation spectroscopy) and hydrodynamic techniques (field flow fractionation, gel permeation and ultracentrifugation).

- **Percent Drug Encapsulation:**

The amount of drug encapsulated/ entrapped in liposome vesicle is given by percent drug encapsulation. Column chromatography can be used to estimate the percent drug encapsulation of liposomes. The formulation consists of both free (unencapsulated) and encapsulated drug. So as to know the exact

amount of drug encapsulated, the free drug is separated from the encapsulated one. Then the fraction of liposomes containing the encapsulated drug is treated with a detergent, so as to attain lysis, which leads to the discharge of the drug from the vesicles into the surrounding medium.This exposed drug is assayed by a suitable technique which gives the percent drug encapsulated from which encapsulation efficiency can be calculated. Trapped volume per lipid weight can also give the percent drugencapsulated in a liposome vesicle. It is generally expressed as aqueous volume entrapped per unit quantity of lipid, µl/µmol or µg/mg of total lipid. In order to determine the trapped volume, various materials like radioactive markers, fluorescent markers and spectroscopically inert fluid can be used. Radioactive method is mostly used for determining trapped volume. It is determined by dispersing lipid in an aqueous medium containing a non-permeable radioactive solute like inulin. Alternatively, water soluble markers like 6-carboxyfluorescein, 14C or 3H-glucose or sucrose can be used to determine the trapped volume. A novel method of determining intra vesicular volume by salt entrapment was also reported in literature.

- **Vesicle shape and lamellarity:**

Various electron microscopic techniques can be used to assess the shape of the vesicles. The number of bilayers present in the liposome, i.e., lamellarity can be determined using freeze fracture electron microscopy and 31P-Nuclear magnetic resonance analysis64. Apart from knowing the shape and lamellarity, the surface morphology of liposomes can be assessed using freeze-fracture and freeze-etch electron microscopy.

- **Phospholipid identification and assay:**

The chemical components of liposomes must be analyzed prior to and after the preparation. Barlett assay, Stewart assay and thin layer chromatography can be used to estimate the phospholipid concentration in the liposomal formulation. A spectrophotometricmethod to quantify total phosphorous in a sample was given in literature, which measure the intensity of blue color developed at 825 nm against water. Cholesterol oxidase assay or ferric perchlorate method and Gas liquid chromatography techniques can be used to determine the cholesterol concentration.[45]

- **Conclusion:-**

Liposomes are one of the unique novel drug delivery system, which have potential use in controlled & targeted drug delivery. Liposomes can be administered orally, parentally & topically and also used in cosmetic &hair technologies, sustained release formulations, diagnostic purpose. These are good carriers in gene delivery. In recent years liposomes are widely used as carriers for targeted drug delivery. Drugs encapsulated in liposomes can have a significantly altered pharmacokinetics. The efficacy of the liposomal formulation depends on its ability to deliver the drug molecule at the site of action over a prolonged period of time, simultaneously

reducing its (drug's) toxic effects. The drugs are encapsulated within the phospholipids bilayer and are expected to diffuse out from thebilayer slowly. Various factors like drug concentration, drug to lipid ratio, encapsulation efficiency and in vivo drug release must be considered during the formulation of liposomal drug delivery systems. Finally, liposomal drugs exhibit reduced toxicities and retain enhanced efficacy compared with free complements.

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