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# A Review on Liposomes : A Promising Technique for Drug Delivery System

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ABSTRACT

This Liposomes are microscopic (unilamellar or multilamellar) vesicles. Liposomes have been considered promising and versatile drug vesicles. Compared with traditional drug delivery systems, liposomes exhibit better properties, including site-targeting, sustained or controlled release, protection of drugs from degradation and clearance, superior therapeutic effects, and lower toxic side effects. Given these merits, several liposomal drug products have been successfully approved and used in clinics over the last couple of decades. Currently, a number of liposomal formulations are on the marker for cancer treatment and many more are in pipe line. This review discusses about the liposome components, methods of preparation, drug encapsulation mechanism and the potential therapeutic applications of liposomes in cancer therapy. The term liposome means lipid body. It has been derived on the basis of name of subcellular particles, ribosome. Liposomes were first made by A.D Bangham in early 1960s. Their size ranges from 25 to 500 nm. This paper summarizes exclusively scalable techniques and focuses on limitations in respect to industrial applicability and regulatory requirements concerning liposomal drug formulations based on FDA and EMEA documents.

**Keywords:** Liposomes, Microscopic, Drug Delivery, Versatile drug Vesicles, Cancer Therapy.

## I. INTRODUCTION

This Liposomes are self-assembled (phospho)lipid-based drug vesicles that form a bilayer (uni-lamellar) and/or a concentric series of multiple bilayers (multilamellar) enclosing a central aqueous compartment[1]. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery. Liposome properties differ

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considerably with lipid composition, surface charge, size, and the method of preparation[5,6,7]. The size of liposomes ranges from 30 nm to the micrometer scale, with the phospholipidbilayer being 4–5 nm thick[2]. The field of liposomology was launched by the British scientist Alec Bangham and colleagues at Babraham Cambridge in the mid-1960s[3], and they first published the structure of liposome sin 1964[4]. The choice of bilayercomponents determines the 'rigidity' or 'fluidity' and thecharge of the bilayer. For instance, unsaturated phosphatidylcholine species from natural sources (egg orsoybean phosphatidylcholine) give much more permeable and less stable bilayers, whereas the saturated phospholipids with long acyl chains (for example, dipalmitoylphos phatidylcholine) form a rigid, rather impermeable bilayerstructure[5,6,7].

Tumor cells may overexpress specific receptors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), folic acid (FA), integrin, CD44 (a cell surface glycoprotein), CD13, and prostate-specific membrane antigen[8]. Liposomes are extensively used as carriers for numerous molecules in cosmetic and pharmaceutical industries. Additionally, food and farming industries have extensively studied the use of liposome encapsulation to grow delivery systems that can entrap unstable compounds (for example, antimicrobials, antioxidants, flavors and bioactive elements) and shield their functionality. Liposomes can trap both hydrophobic and hydrophilic compounds, avoid decomposition of the entrapped combinations, and release the entrapped at designated targets[9-11]. Cancer is the major health concern of the century because of the leading cause of death worldwide[18,19]. It kills millions of people very year and its burden continues to rise at an alarming rate globally. Cancer is the uncontrolled growth of cells, which occurs due to the accumulation of genetic mutations and aberrant signaling of various pathways related to the growth and survival of the cells[20-23]. The complexity at genetic and phenotypic levels in cancer cells leads to the clinical diversity and therapeutic resistance in cancer cells. Chemotherapy is most commonly used treatment among a variety of approaches currently being used for the treatment of cancer, which, however, possesses several limitations and side effects[24-26]. According to an estimate, more than 90% cancer drugs exhibit poor bioavailability and pharmacokinetics[26]. Therefore, there is a prerequisite to develop appropriate drug delivery systems, which can improve the bioavailability, pharmacokinetic properties and can deliver the active drug molecules to the site of action, without affecting the healthy cells. To overcome the limitations of conventional chemotherapy, a number of nanocarrier delivery systems have been developed and extensively used for drug delivery to cancer cells[27-29].

Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs[12] and simplify site-specific drug delivery to tumor tissues[13], liposomes have increased rate both as an investigational system and commercially as a drug delivery system. Many studies have been conducted on liposomes with the goal of decreasing drug toxicity and/or targeting specific cells[14-16]. Liposomal encapsulation technology (LET) is the newest delivery technique used by medical investigators to transmit drugs that act as curative promoters to the assured body organs. LET is a method of generating sub-microscopic foams called liposomes, which encapsulate numerous materials[17].

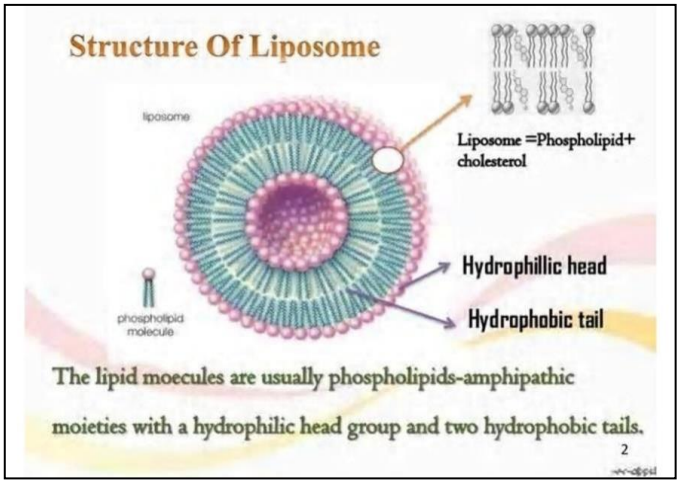



**Fig.1.** Structure of liposome

## ADVANTAGES OF LIPOSOME

- Stability increased if liposome prepared via encapsulation.
- Liposomes increased efficacy and therapeutic index of drug (actinomycin-D).
- Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol).
- Liposomes help reduce the exposure of sensitive tissues to toxic drugs.
- Site avoidance effect.
- Liposomes are flexible, non-toxic, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations.
- Flexibility to couple with site-specific ligands to achieve active targeting.
- Suitable for delivery of hydrophobic, amphipathic and hydrophilic drugs.
- Protect the encapsulated drug from the external environment.
- Reduced toxicity and increased stability-As therapeutic activity of chemotherapeutic agents can be improved through liposome encapsulation. This reduces deleterious effects that are observed at conc. similar to or lower than those required for maximum therapeutic activity.
- Reduce exposure of sensitive tissues to toxic drugs.

## DISADVANTAGES OF LIPOSOME

- Short half-life.
- Low solubility.
- Leakage and fusion of encapsulated drug/molecules.
- Production cost is high.
- Fewer stables.
- Sometimes phospholipids undergo oxidation and hydrolysis-like reaction.

## MARKETED LIPOSOMAL PRODUCTS

The Liposomal Products that are sold14 different liposomal product types have been approved, according to the FDA and EMA's approved medicine database, which we searched. Note that generics, lipid complexes (such as Abelcet, Aphotic, and Nonpatrol), and liposomal medicines with national authorization in Europe are not included in this list. The FDA authorized Doxil, an injectable containing doxorubicin hydrochloride, as the first liposomal medicine in 1995.43% of these marketed products were authorized before to 2000, and 57% of these products were approved prior to 2010. The therapeutic field includes lung illness, anaesthesia, infection, vaccination, and photodynamic treatment in addition to its primary focus on cancer therapy. The two primary dosage forms under consideration are lyophilization powder and sterile suspension. The methods of administration include of oral inhalation, local infiltration, epidural, intrathecal, and intravenous infusion.

| Product | Drug | Company |
|---|---|---|
| Ambisome™ | Amphotericin B | NeXstar Pharmaceuticals, Inc., CO |
| Abelcet™ | Amphotericin B | The Liposome Company, NJ |
| Amphocil™ | Amphotericin B | Sequus Pharmaceuticals, Inc., C.A. |
| Doxil™ | Doxorubicin | Sequus Pharmaceuticals, Inc., C.A. |
| DaunoXome™ | Daunorubicin | NeXstar Pharmaceuticals, Inc., CO |
| MiKasome™ | Amikacin | NeXstar Pharmaceuticals, Inc., CO |
| DC99™ | Doxorubicin | Liposome Co., NJ, USA |
| Epaxel™ | Hepatitis A Vaccine | Swiss Serum Institute, Switzerland |
| ELA-Max™ | Lidocaine | Biozone Labs, CA, USA |

**Table 1 :** Various Marketed Formulations of Liposomes

## MECHANISM OF LIPOSOME FORMATION

The main cause for bilayer formation in liposomes is the amphiphilic nature of phospholipids. These molecules have a hydrophilic head and a hydrophobic tail. When they are dispersed in an aqueous medium, they organize themselves in a way that the hydrophilic head faces outwards towards the water, while the hydrophobic tails face each other. This arrangement leads to the formation of lamellar sheets, and eventually, spherical vesicle-like structures known as liposomes. The process of bilayer formation is facilitated by the input of energy through methods such as sonication, shaking, heating, homogenization, etc. This energy input promotes interactions between lipid molecules and between lipids and water

molecules. These interactions lead to the formation of bilayer vesicles, which reach a thermodynamic equilibrium in the aqueous phase. This bilayer structure is fundamental to the properties and functions of liposomes, allowing them to encapsulate both hydrophilic and hydrophobic substances within their aqueous core and lipid bilayer respectively. This property is the basis for their applications in drug delivery, cosmetics, and various other fields.

1. The unfavourable interaction generated between hydrophilic and hydrophobic phase can be minimized or decreased by folding into closed concentric vesicles.
2. Large bilayer vesicle formation assists the reduction of large free energy difference present between the hydrophilic and hydrophobic environment.
3. Maximum stability to super molecular self-assembled structure can be attained by forming into vesicles.

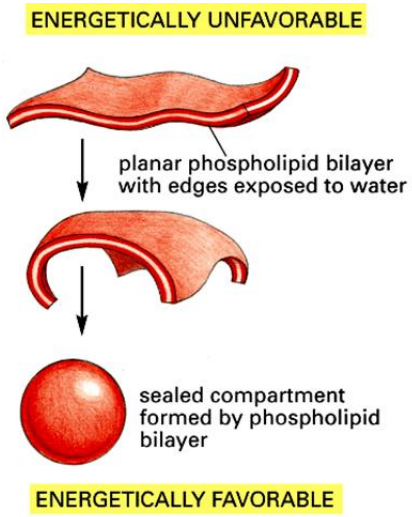



**Fig 2 :** Mechanism of formation of liposome

## TYPES OF LIPOSOMES[30-35]

Liposomes are classified on the basis of

### A) BASED ON STRUCTURAL PARAMETERS:

**1. Unilamellar vesicles:**

- Small unilamellar vesicles (SUV): size ranges from 20-40 nm
- Medium unilamellar vesicles (MUV}: size ranges from 40-80 nm.
- Large unilamellar vesicles (LUV): size ranges from 100nm-1,000 nm

**2. Oligolamellar vesicles (OLV):**

These are made up of 2-10 bilayers of lipids surrounding a large internal volume

**3. Multilamellar vesicles (MLV):**

They have several bilayers. They can compartmentalize the aqueous volume in an infinite number of ways. They differ according to way by which they are prepared. The arrangements can be onion like arrangements of concentric spherical bilayers of LUV/MLV enclosing a large number of SUV etc.

### B) BASED ON METHOD OF LIPOSOME PREPARATION:

**1. REV:**

Single or oligolamellar vesicles made by Reverse-Phase Evaporation Method.

**2. MLV-REV:**

Multilamellar vesicles made by Reverse-Phase Evaporation Method.

**3. SPLV:**

Stable Pluriglandular Vesicles.

**4. FATMLV:**

Frozen and Thawed MLV.

**5. VET:**

Vesicles prepared by extrusion technique

**6. DRV:**

Dehydration-rehydration method.

### C) BASED UPON COMPOSITION AND APPLICATION:

**1. Conventional Liposomes (CL):**

Neutral or negatively charged phospholipids and Cholesterol.

**2. Mucogenic Liposomes (RSVE):**

Reconstituted Sendai virus envelopes

**3. pH sensitive Liposomes:**

Phospholipids such as PE or DOPE with either CHEMS or OA

**4. Cationic Liposomes:**

Cationic lipids with DOPE

**5. Long Circulatory (Stealth) Liposomes (LCL):**

They have polyethylene glycol (PEG) derivatives attached to their surface to decrease their detection by phagocyte system (reticuloendothelial system; RES). The attachment of PEG to liposomes decreases the clearance from blood stream and extends circulation time of liposomes in the body. The attachment of PEG is also known as pegylation.

**6. Immuno-Liposomes:**

CL or LCL with attached monoclonal antibody or recognition sequence.

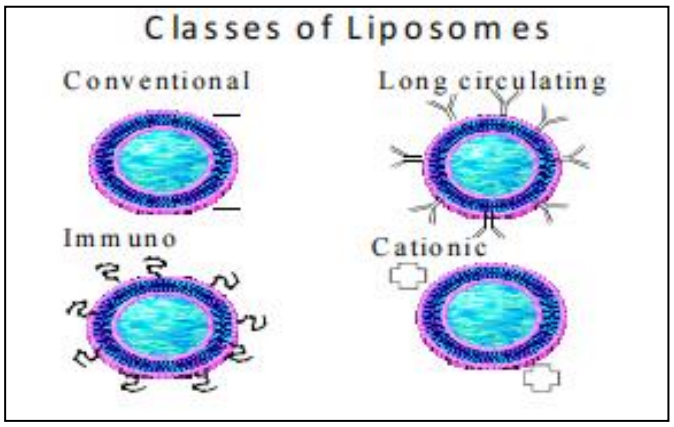



**Fig 3 : Classes of Liposomes**

## STRUCTURE OF LIPOSOME

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) (Fig 4)[36]. Although OLVs and MLVs have an onion-like structures, they also have two to five or more concentric lipid bilayers, respectively. In contrast to MLVs, MVLs have a structure resembling a honeycomb and contain hundreds of non-concentric aqueous chambers surrounded by a single bilayer lipid membrane[37]. Small unilamellar vesicles (SUVs, 30-100 nm), large unilamellar vesicles (LUVs, >100 nm), and giant unilamellar vesicles (GUVs, >1000 nm) are the three additional classifications of ULVs based on particle size[38].There have been reports of ULVs in two different size ranges: SUVs less than 200 nm and LUVs between 200 and 500 nm[39].

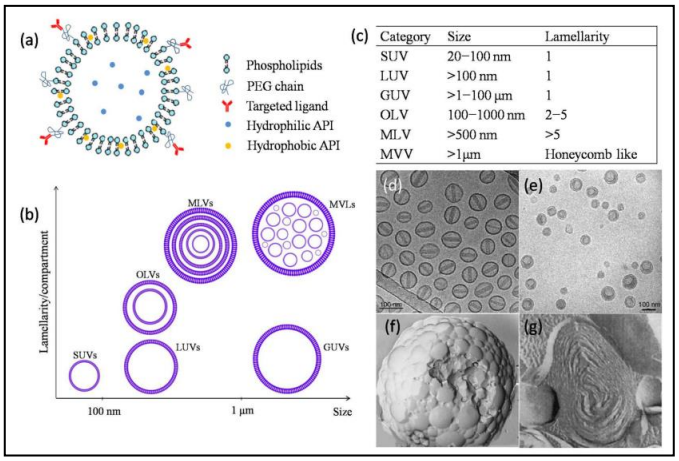



**Fig 4 : The classification and organization of liposomal drug delivery system[40]**

(a) A structural representation of the makeup of liposomes. A typical phospholipid bilayer's size is 4.5 nm, which is significantly smaller than that of the inner aqueous core.

(b) liposomal vesicles' classification based on their lamellarity/compartment and particle size;

(c) the size and lamellarity of various liposome types.

(d, e) the cryo-transmission electron microscopy of Doxil and Vyxeos.

(f, g) electron micrographs of DepoFoamTM particles, which have a typical diameter of 1–100 µm (e.g., DepoCyt) and MLVs, which have a typical diameter of 0.2–5 µm (e.g., Mepact).

## MAIN COMPONENTS OF LIPOSOMES

The major components of liposomes are phospholipids and cholesterol, major constituents of natural bio membranes. The chemical properties of these lipids control the behaviour of liposomes.

### Phospholipids

The most popular phospholipids for liposome synthesis are synthetic phosphatidylcholine (PC) and natural phosphatidylcholine (found in eggs, soy, or other natural sources). Natural phospholipids, such those found in eggs or soyabeans, are less stable than

their manufactured counterparts because they contain higher concentrations of polyunsaturated fatty acids. The molar percentage of phospholipids varies from 55 to 100% of total liposomal components. 2-distearoyl-sn-glycerophosphocholine (DSPC) is the most often occurring phospholipid found in liposomes. Fig 5A displays the DSPC's chemical structure. This molecule is made up of hydrocarbon chain-based hydrophobic part and polar phosphate head group. The polar head creates the liposomes' shell, while the hydrocarbon chains make up their inside. A functional group can be attached to alter the head part. Functional phospholipids, such as 1,2-distearoylsn-glycero-3-phosphoethanolamine (DSPE), are utilized to conjugate other polymers, such as polyethylene glycol (PEG) Fig 5B. The final form and dimensions of the liposomes are determined by the kind, molar proportion, and packing orientation of phospholipids. The length of lipid molecules and the number of head groups determine the orientation of phospholipids in the liposome bilayer[41-44]. More stable bilayers are produced when phospholipids with higher phase transition temperatures are used[46]. This lessens the chance of early encapsulated component leakage, but care must be taken to make sure that once encapsulated medications reach the target site of action, they can still escape the liposomes. However, denaturation of the pharmaceuticals contained during the loading or scaling operations may happen if the phase transition temperature of the chosen phospholipids is too high. Thus, a fair balance needs to be achieved to ensure that the phase transition temperatures of the chosen lipids avoid early component leaking while allowing processing to happen at temperatures that are safe for all liposomal components[41,42,45].

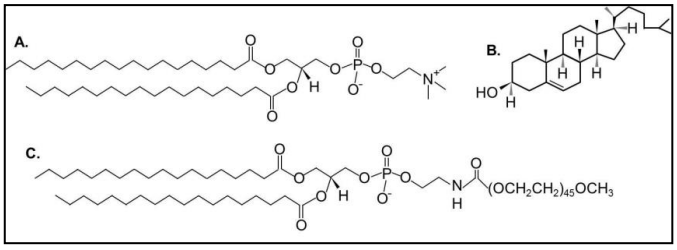



**Fig 5 : Chemical structures of common liposomal components**

(A) 1, 2-distearoyl-sn-glycerophosphocholine (DSPC)
(B) Cholesterol
(C) 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine polyethylene glycol (DSPEPEG).

## Cholesterol

Leaky liposomes are produced by the rotational freedom caused by phospholipid flip-flop motions. The primary ingredient added to liposomal formulations to stabilize the liposome bilayer is cholesterol. As cholesterol gives liposomes membrane fluidity, elasticity, permeability, and stability, its molar percentage varies from 30 to 45 percent of all liposome components depending on the rigidity and fluidity of the bilayer. Fig 5C shows the chemical structure of cholesterol[47,48]. Additionally, cholesterol gives liposomes their stiffness by preventing lipid bilayers from going through a phase transition, which lowers the number of medications that are encapsulated from leaking out. Thus, the final phase transition temperature of the bilayer is likewise influenced by the proportion of cholesterol utilized in the liposome manufacturing process. According to certain research, cholesterol may also aid in shielding the lipid bilayer from hydrolytic breakdown. In addition to phospholipid and cholesterol, numerous additional ingredients have also been employed, depending on the eventual use of liposomes. Liposomes can be positively charged, negatively charged, or neutrally charged depending on the component employed. The liposomes' surface charge is a significant factor in determining their use and

destiny. The other often utilized liposome component is PEG, which is usually used to speed up blood circulation due to its covert nature and wide range of uses.

## DESIGN AND DEVELOPMENT OF LIPOSOMES

The ultimate identity of any liposomal system and hence its properties are determined by the various factors. All these variables, directly or indirectly, have their effect on the formation of liposomes. Therefore, it is necessary that these variables must be carefully controlled during the design of liposomes. In other words, during the formulation of liposomes all these variables must be optimized in order to obtain the best possible formulation with maximum stability and entrapment efficiency. The design of drug delivery system should always be taken from the past biology of the system. For example, the anticancer drugs are targeted using liposomes to the specific vascular structure of tumor tissue. Another example includes the use of liposomes to target the drug to liver and spleen in leishmaniasis, as particulate uptake by liver and spleen is a known fact. Once a correlation is obtained between the liposomal surface and the resulting biological response, more specific forms of targeting that involve the incorporation of molecular recognition elements may be undertaken. Stealth liposomes having a coating of polyethylene glycol have been widely exploited for the tumor targeting as they have low uptake by reticule-endothelial system and spleen. In the similar manner, the pharmacodynamics and pharmacokinetics of liposomes can be changed by inclusion of charge inducers and steric stabilizers like dicetylphosphate (DCP), stearylamine (SA), solulan C-24 etc. Some of these factors are shown below in Fig 6.

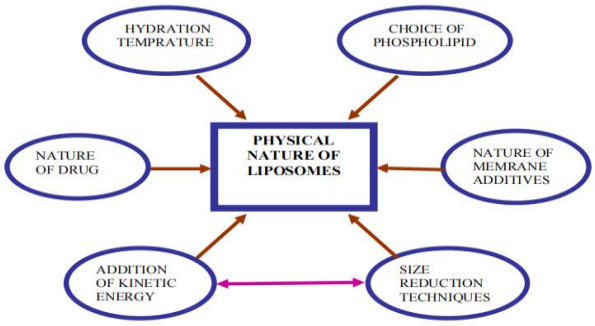



**Fig 6 : Factors effecting the formation of liposomes**

## PREPARATION OF LIPOSOMES[52-59]

The preparation of all types of vesicular systems requires the input of energy[49].

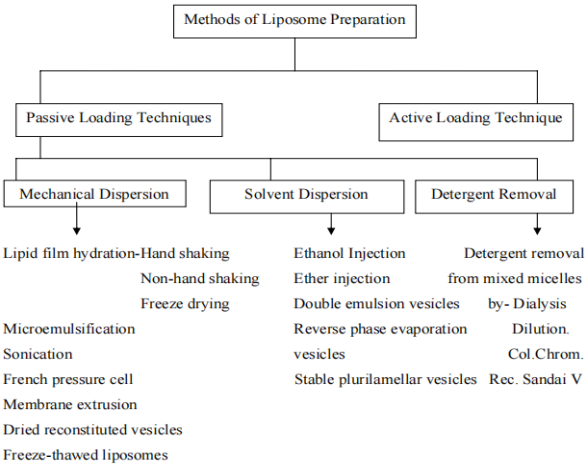
### General methods of preparation

All the methods of preparing the liposomes involve three basic stages:

1. Drying down of mixture of lipids from an organic solvent.
2. Dispersion of lipids in aqueous media.
3. Separation and purification of resultant liposome[50].

The various methods of preparation of liposomes are as under[51].

### Method of liposome preparation and drug loading




### Mechanical Dispersion

The water quantities included in lipid membranes in these procedures range from 5 to 10%, which is a

relatively tiny percentage of the overall volume utilized for preparation. During preparation, a significant quantity of the water-soluble medication is squandered. However, a substantial percentage of lipid-soluble drugs may be encapsulated. These techniques result in the formation of MLVs; further processing is necessary to create unilamellar vesicles.

**Lipid film hydration: -**

- **Hand shaking:**

This is the most straightforward and popular approach. A 250 ml round-bottom flask is filled with the lipid mixture and charged components after they have been dissolved in a 2:1 ratio of methanol and chloroform. The flask rotates at 60 rpm and is fixed to a rotating evaporator that is coupled to a vacuum pump. Around 30 degrees cause the organic solvents to evaporate. Following the formation of a dry residue at the flask's walls, spinning is kept up for fifteen minutes. Nitrogen is injected into the evaporator once it is disconnected from the vacuum pump. To remove any remaining solvent, the flask is then taken out of the evaporator and put onto a lyophilized. Next, nitrogen and five millilitres of phosphate are added to the flask once again. After that, 5 ml of phosphate buffer is added and the flask is once more flushed with nitrogen. Once more, the flask is linked to the evaporator and spun at a speed of around 60 revolutions per minute for 30 minutes, or until the flask wall is completely free of lipid. Finally, a creamy white suspension form. For the purpose of providing MLVs, the suspension is let to stand for two hours to finish the swelling process.

- **Non-Hand shaking:**

With the exception of the careful swelling operation, this is comparable to the shaking approach. The conical flask's flat bottom is covered with the lipid solution in a combination of methanol and chloroform. Nitrogen passes through the flask and evaporates the solution at normal temperature without affecting it. Nitrogen that has been soaked with water is poured through the flask until the dried film's opacity is completely gone. Lipid is inflated by adding bulk liquid after it has been hydrated. The flask is tilted to one side. Ten to twenty millilitres of 0.2M sucrose dissolved in distilled water are then added to the flask's side, and the flask is gradually raised back to its upright position. The lipid layer is exposed to the solvent gradually. The lipid layer at the flask's bottom is allowed to be slowly covered by the solution. The flask is capped, flushed with nitrogen, and let to stand at 37 degrees for two hours to allow for swelling. The vesicles are then combined to create a milky solution. For ten minutes, the suspension is centrifuged at 1200 rpm. It removes the layer of MLVs that were floating on the surface. Liquid remaining after processing yields LUVs.

- **Freeze drying:**

Freeze-drying the lipid dissolved in an appropriate organic solvent is another way to disperse the lipid in a finally split state before adding aqueous medium. Tertiary butanol is often utilized as the solvent. Every procedure listed above yields MLVs. These are either too big or too dispersed. The following steps are used to further process the produced MLVs in order to change their size.

- **Micro-emulsification of liposomes: -**

A device known as a micro fluidizer is used to create concentrated lipid solution into tiny vesicles. Large MLV suspensions of the lipids can be added to the fluidizer. Via a 5-micrometer screen, the fluid is pumped through this apparatus at extremely high pressure. After that, it is driven via lengthy microchannels, causing two streams of fluid to clash quickly and at a right angle. After the collected fluid is circulated through the pump and interaction chamber, spherically-shaped vesicles can be obtained.

- **Sonification:**

By using this technique, the vesicles get smaller and the lipid solution gets more energy. The MLV can be

subjected to ultrasonic irradiation to accomplish this. Two sonication techniques exist: 1) using a bath sonicator, and 2) using a probe sonicator. For suspensions that demand a high energy in a compact volume, the probe sonicator is employed. (For instance, a viscous aqueous phase or a high concentration of lipids) For a high volume of diluted lipids, the bath sonicator is utilized. One drawback of the probe sonicator is that the metal tip may contaminate the preparation. Small unilamellar vesicles are created with this technique, then ultra centrifugation is used to purify them.

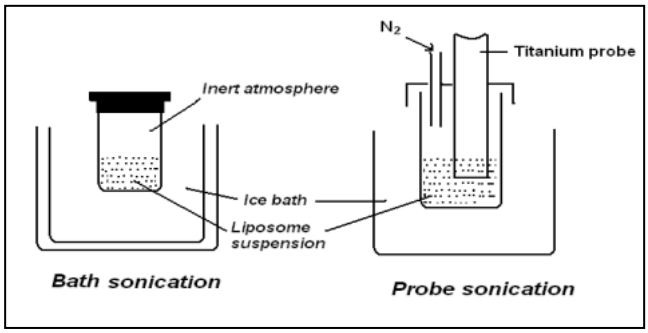



**Fig 7 : Sonification Apparatus**

- **Extrusion of Membranes:**

Using a membrane filter with a set pore size, the size is decreased in this manner. Membrane filters come in two varieties. The types of nucleation tracks and winding paths. Sterile filtering is carried out using the former. The fibres that form the cross are randomly arranged in this path. The density of fibres within the matrix regulates the average diameter of these fibres. When one tries to transmit liposomes across such a membrane, they are hit since their size exceeds the channel diameter. Polycarbonate sheets that are thin and continuous make up the nucleation track type. They will offer less resistance to passage of liposomes as these consist of straight sided pore holes ofexact diameter bored from one side to another. This method can be used to process both LUVs and MLVs.

- **Freeze-thawed liposomes :**

This technique involves rupturing and rejecting SUVs while the solute equalizes between the interior and outside. The entrapment efficiency and volume are increased by this technique. Vesicles between lamellae and inside vesicles will occur as a result of this technique. This technique can result in a 30% increase in entrapment volume.

- **Solvent Dispersion**

These procedures include dissolving lipids in an organic solution initially, followed by interaction with an aqueous phase that contains the components that the liposome is to entrap. The phospholipids align themselves to create a monolayer at the interface between the aqueous and organic phases, which is a crucial step in the formation of the liposome bilayer.

- **Ethanol Injection:**

This is an easy technique. With this technique, a fine needle is used to quickly inject an ethanol solution containing the lipids into an excess of saline or another aqueous media. The phospholipid molecules are uniformly distributed throughout the media once the ethanol is diluted with water. A large percentage of SUVs with a diameter of around 25 nm are produced by this process.

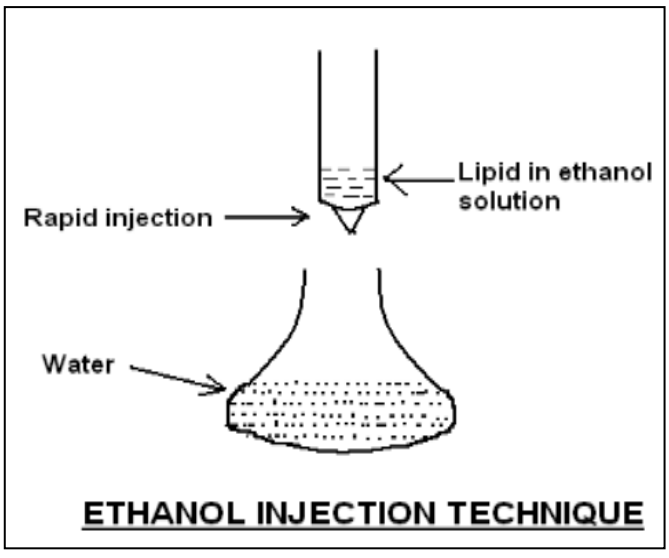



**Fig 8 : Ethanol Injection**

- **Ether Injection:**

At 55°C to 65°C, or at lower pressure, a lipid solution mixed in diethyl ether or an ether-methanol mixture

is progressively injected into an aqueous solution of the substance to be encapsulated. Thus, ether is removed under vacuum, which results in the formation of liposomes. The technique's primary drawbacks are the population's heterogeneity (ranging from 70 to 200 nm) and the compounds' exposure to organic solvents at high temperatures.

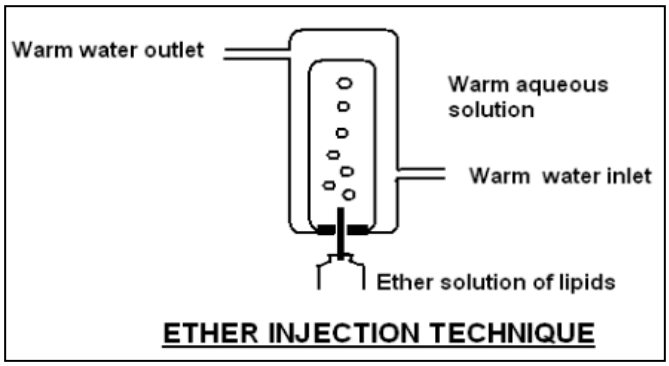



**Fig 9: Ether Injection**

- **Reverse phase evaporation vesicles: -**

Since it made it possible to create liposomes with a high aqueous space-to-lipid ratio and the capacity to entrap a significant portion of the aqueous material given, this approach advanced liposome technology. The formation of inverted micelles is the foundation of reverse-phase evaporation. The formation of these inverted micelles occurs by the sonication of a mixture including an organic phase that solubilizes the amphiphilic molecules and a buffered aqueous phase that includes the water-soluble molecules to be encapsulated into the liposomes. These inverted micelles become thick and gel-like as a result of the gradual removal of the organic solvent. This process reaches a crucial stage where the gel state collapses, disrupting part of the inverted micelles. Liposomes are produced when an overabundance of phospholipids in the environment contributes to the production of a full bilayer around the remaining micelles. In comparison to hand-shaken or multilamellar liposomes, reverse phase evaporation liposomes have an aqueous volume-to-lipid ratio four times greater and may be produced from a variety of lipid formulations.

- **Detergent Removal**

This method also called as removal of non-encapsulated material. Through their association with phospholipid molecules, detergents in this approach bring the phospholipids into intimate contact with the aqueous phase. Micelles are the structures that arise from this interaction. They are made up of millions upon millions of individual molecules. CMC is the detergent concentration in water at which micelles begin to form. The detergent molecule is in free solution below CMC. Large volumes of micelle develop when the detergent molecule dissolves in water at concentrations greater than the CMC. More detergent is integrated into the bilayer when the concentration of detergent injected is raised, up until a point at which the bilayer converts from lamellar to spherical micellar form. The size of the micelles decreases with additional increases in detergent concentration. Detergent removal from mixed micelles by some below method.

- **Dialysis: -**

Lipids have been solubilized by detergents at their critical micelle concentrations (CMC). The micelles get better at phospholipid as the detergent separates from them, and eventually they unite to form LUVs. Dialysis was used to get rid of the detergents. For the removal of detergents, a commercial equipment known as LipoPrep (Diachema AG, Switzerland) is available. It is a dialysis system version. Dialysis can be carried out in equilibrium dialysis, which involves placing the dialysis bags inside large buffers devoid of detergent.

- **Dilution: -**

When detergent and phospholipids are added to an aqueous mixed micellar solution, the micellar size and polydispersity fundamentally increase. As the system is diluted past the mixed micellar phase boundary, a spontaneous transition from poly-dispersed micelles to vesicles takes place.

## ENCAPSULATION OF DRUGS INTO LIPOSOMES

The methods of drug encapsulation in to the liposomes can be divided into two sub groups.

The passive loading in which drug encapsulation occur during the vesicle formation process and the active loading in which drug is entrapped after the formation of vesicles.

### Passive loading[27,28,60-62]

The purpose of passive loading is to encapsulate the medication while liposomes are being formed. By combining with the hydrating buffer used to moisten the thin lipid film during liposome synthesis, the hydrophilic medicines are loaded into the interior core of the liposomes. When a thin dry film of lipids is prepared, lipophilic medicines are combined with other liposome components and injected into lipid bilayers. Drug molecules that are not entrapped are extracted from the liposome solution using gel-filtration chromatography or dialysis.

Lipid concentration, liposome size, lipid selection, etc. all affect encapsulation efficiency. Water-soluble chemicals do not interact with the lipid bilayer; hence their encapsulation effectiveness is proportional to the aqueous volume encapsulated in the liposomes when supplied passively. According to Akbarzadeh et al. (2013), large vesicles will have a better encapsulation effectiveness than tiny vesicles. Conversely, medications that interact with the lipid bilayer, including lipophilic compounds, typically have higher encapsulation rates.

Because of this, a number of techniques have been devised to enhance the effectiveness of encapsulation by attaching a lipophilic chain to the drug molecule, so increasing its lipophilicity and improving its partition into the lipid bilayer. Large vesicles will have a better encapsulation effectiveness than tiny vesicles. Conversely, medications that interact with the lipid bilayer, including lipophilic compounds, typically have higher encapsulation rates. Selecting the right lipid composition is essential for improved loading effectiveness when using this technique. For instance, using a cationic lipid would significantly increase the encapsulation effectiveness when loading strongly negatively charged nucleotide molecules, like antisense or siRNA, because of the improved drug/lipid interaction.

### Active loading[61-65]

Active loading, also known as remote loading, is a technique used to load specific weakly acidic or alkaline medicinal molecules into manufactured liposomes. This action is fuelled by an electrochemical potential generated by the pH or ion gradients formed within the liposomes' lipid bilayer. Using a buffer with a predetermined pH and ion concentration, the pH or ion gradients are produced throughout the liposome production process. After that, the liposomes' external pH is adjusted using a new buffer with a different pH or an ion concentration using size exclusion chromatography or dialysis. To guarantee fluidity and effective transport across the bilayer, the medication is loaded by mixing with liposomes after the pH gradient has been created across the membranes of the liposomes. This is usually done at a temperature higher than the lipids' phase transition temperature. Drug molecules interact and get charged with the ions inside liposomes. The drug molecules that are charged are confined within the liposome core and cannot escape. The best illustration of the active loading by pH gradient approach is DoxilTM, liposomal doxorubicin. Fig 10 illustrates the active loading of doxorubicin using a pH gradient technique.

A pH gradient is produced, as the picture illustrates, when the gradient of citrate buffer liposome is greater than 1000 times that of the medium's citrate buffer. Weak bases like doxorubicin oscillate between ionized and non-ionized states. The latter may penetrate the lipid bilayer, ionize in the high-proton intra-liposome environment, and cause doxorubicin to accumulate inside the liposome with great efficiency. The loading of chloroquine diphosphate into liposomes is a further illustration of the pH-gradient approach. In a different research, liposomes

were also loaded using the pH gradient approach with oxymatrine, the main active alkaloid ingredient that was isolated from the traditional Chinese herb medicine Sophora flavescens, which is used to treat hepatitis B in clinical therapy in China.

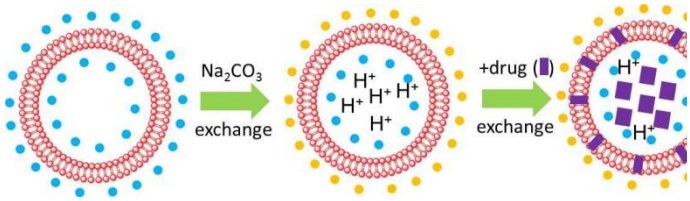



**Fig 10: Active loading of drugs into liposomes**

Liposomes were prepared by hydrating in citrate buffer
( ) and then external phase was exchanged with Na2CO3 ( ) to create a pH gradient. (C) The neutral form of the externally added drug ( ) can cross the bilayer and is protonated ( ) and trapped inside the vesicles.

## PURIFICATION OF LIPOSOMES[66,67]

Centrifugation, dialysis, and gel filtration chromatography are often used to purify liposomes. The most used tool for chromatographic separation is Sephadex-50. Hollow fibre dialysis cartridges can be utilized in the dialysis process. SUVs in regular saline can be isolated using the centrifugation technique by centrifuging at 200000 g for 10–20 hours. Centrifuging at 100,000 g for less than an hour separates MLVs.

## EVALUTION OF LIPOSOMES[67-70]

Liposomal formulation and processing for specified purpose are characterized to ensure their predictable in vitro and in vivo performance. The characterization parameters for purpose of evaluation could be classified into three broad categories which include physical, chemical and biological parameters.

- Physical characterization evaluates various parameters including size, shape, surface features, lamellarity, phase behaviour and drug release profile.
- Chemical characterization includes those studies which establish the purity and potency of various lipophilic constituents.
- Biological characterization parameters are helpful in establishing the safety and suitability of formulation for therapeutic application.

Some of parameters are:

### ➢ Vesicle shape and lamellarity:

Electron microscopic techniques can be used to evaluate the morphology of vesicles. The number of bilayers present in liposomes, or the lamellarity of the vesicles, is ascertained by P-31 Nuclear Magnetic Resistance Analysis and Freeze-Fracture Electron Microscopy.

### ➢ Vesicle size and size distribution:

The literature describes a number of methods for determining size and size distribution. Light microscopy, fluorescent microscopy, electron microscopy (particularly transmission electron microscopy), field flow fractionation, laser light scattering, photon correlation spectroscopy, and gel permeation and gel exclusion are a few of these. Since electron microscopy allows one to observe individual liposomes and gain accurate information about the liposome population's profile throughout the whole range of sizes, it is the most precise approach for determining liposome size. Regretfully, it takes a lot of time and requires tools that aren't always readily available. The laser light scattering method, on the other hand, is faster and easier to use, but it has the drawback of only being able to measure the average property of the liposome mass. These techniques all need very expensive equipment. Gel exclusion chromatography methods are advised if just an approximate understanding of the size range is needed, as the only costs involved are those of the gel material and buffers. The form, size, and stability of

liposomes have been investigated using atomic force microscopy, another more modern microscopic method. The majority of techniques employed in size, shape, and distribution study fall into one of four categories: hydrodynamic, diffraction, microscopic, or scattering.

- **Microscopic Techniques**
  - **Optical Microscopy:**

The microscopic approach, which uses a fluorescent microscope, phase contrast microscope, and bright-field microscope, is helpful in determining the size of big vesicles.

- **Negative Stain TEM:**

Microscopic Electron The two major methods used to evaluate the size and form of liposomes are scanning electron microscopy and negative-stain TEM. The latter method is not as favoured. Bright spots are visible against a dark backdrop using negative stain electron microscopy, which is why it is called negative stain.

Uranyl acetate, phosphotungstic acid (PTA), or ammonium molybdate are the negative stains utilized in TEM investigation. Uranyl acetate is cationic in nature, whereas PTA and ammonium molybdate are anionic.

- **Cryo-Transmission Electron Microscopy Techniques (cryo-TEM):**

This technique has been used to elucidate the surface morphology and size of vesicles.

- **Diffraction and Scattering Techniques**
  - **Laser Light Scattering:**

The examination of the temporal dependence of intensity variation in scattered laser light caused by Brownian motion of particles in solution or suspension is known as photon correlation spectroscopy, or PCS. Small particles disperse more quickly than big particles, hence the pace at which the intensity of scattered light fluctuates varies as well. As a result, the translational diffusion coefficient (D) may be calculated. The Stoke-Einstein equation can then be used to calculate the mean hydrodynamic radius (Rh) of the particles. This method allows one to measure particles down to roughly 3 nm in size.

- **Hydrodynamic Techniques:**

This method uses an ultracentrifuge and gel permeabilization. A technique called exclusion chromatography was used on large pure gels to distinguish between radial MLVs and SUVs. Large vesicles, with a diameter of 1-3 μm, typically do not enter the gel and remain at the top of the column instead. A quick and convenient method for estimating the size distribution of liposome production is the thin layer chromatography system with agarose beads. Nevertheless, it was not mentioned if this method, unlike the more traditional column chromatography, was susceptible to a physical obstruction of the agarose gel's pores.

➢ **Encapsulation Efficiency and Trapped Volume**

These determine amount and rate of entrapment of water-soluble agents in aqueous compartment of liposomes.

- **Encapsulation Efficiency:**

It is commonly represented as % entrapment/mg lipid and represents the percentage of the aqueous phase, and consequently the percentage of water-soluble medication, that is finally imprisoned during the creation of liposomes. Two methods are used to evaluate encapsulation efficiency: the proton aggregation method and the Mini column centrifugation method. On a small scale, Mini column centrifugation is typically utilized for liposome separation and purification. The hydrated gel is put into a barrel of a 1 ml syringe without a plunger and plugged with a Whatman GF/B filter pad in the tiny column centrifugation technique. Within a centrifuge tube, this barrel is resting. To drain the surplus saline solution from the gel, spin this tube for three minutes at 2000 rpm. The gel column ought to have dried and separated from the barrel's side after centrifugation. The collecting tube's eluted saline is then taken out. After adding 0.2 ml of liposome solution dropwise to

the gel bed's top, the column is spun for three minutes at 2000 rpm to release the liposome-containing void volume into a centrifugation tube. After that, the elute is taken out and placed aside for assay. Liposomes that are negatively and neutrally charged can be made using the protease aggregation approach.

- **Trapped volume:**

It is a crucial factor that controls the shape of vesicles. The aqueous entrapment volume per unit quantity of lipids is known as the trapped or internal volume. Between 0.5 and 30 microliters/micromole may be used. To calculate trapped/internal volume, a variety of materials are utilized, such as fluorescent markers, radioactive markers, and spectroscopically inert fluid. The best method for determining internal volume is to measure the amount of water directly using nuclear magnetic resonance (NMR) measurement of the water signal after substituting the external medium (water) with a spectroscopically inert fluid (deuterium oxide). By scattering lipid in an aqueous solution containing a non-permeable radioactive solute, trapped volume may also be empirically assessed. Centrifugation is used to remove external radioactivity, and residual activity per lipid is then calculated to indicate the amount of solute trapped.

- **Phase Response and Transitional Behaviour**

Studies are conducted on the phase transitions shown by liposomes and lipid bilayers in relation to stimulus-mediated fusion of liposomal components with target cells or drug release. Since the phase behaviour of the liposomal membrane determines properties like permeability, fusion, aggregation, and protein binding, an understanding of phase transitions and the fluidity of phospholipid membranes is crucial for both the production of liposomes and their use. Freeze fracture electron microscopy has been used to assess the phase transition. Through the use of differential scanning colorimeter (DSC) examination, they are more thoroughly confirmed.

- **Drug Release**

An accurately calibrated in vitro diffusion cell can be used to evaluate the process of drug release from liposomes. Using in vitro tests to estimate drug pharmacokinetics and bioavailability prior to utilizing expensive and time-consuming in vivo research can help the liposome-based formulation. The pharmacokinetic performance of liposomal formulations was predicted by the dilution-induced drug release in buffer and plasma. The bioavailability of the drug was evaluated by an additional assay that measured intracellular drug release caused by liposome degradation in the presence of mouse liver lysosome lysate.

## TARGETING OF LIPOSOMES[66,70-74]

Two types of targeting

- **Passive targeting:**

Such liposomes, which are typically delivered, have been demonstrated to be quickly removed from the bloodstream and absorbed by the RES in the liver and spleen as a passive targeting method. Therefore, when liposomes are intended for macrophages, their capability can be utilized. The effective transport of liposomal antimicrobial drugs to macrophages serves as evidence for this. Antigens are now targeted to macrophages using liposomes as the initial step in the index of immunity. For instance, when liposomal antigen was administered intravenously to rats, the spleen phagocytes mediated the antibody response, but the non-liposome related antigen did not elicit an antibody response.

- **Active targeting:**

Targeting requires that the targeting agents be placed on the liposomal surface in a way that allows for a plug-and-socket-like contact with the target, or receptor. The liposome is physically prepared such that, when the membrane forms, the lipophilic portion of the connection is fixed into the membrane. the liposome's hydrophilic portion, to which the targeting chemical needs to be kept sterically properly in order to attach to the cell surface receptor. The utilization of active targeting can result in

- **Immune liposomes:**

These are either regular or covert liposomes that have antibodies or another type of recognition sequence (such as glycoprotein or other carbohydrates) attached. The liposome is directed to certain antigenic receptors on a given cell by the antibody that is linked to it. Glycolipid or glycoprotein components of the cell surface that are involved in adhesion and cell-to-cell recognition.

- **Magnetic liposomes:**

Hold magnetic iron oxide inside. An external magnetic field that vibrates can be used to guide these liposomes at the delivery locations.

- **Temperature or heat sensitive liposomes:**

They are designed to have a transition temperature that is somewhat higher than body temperature. Once at the location, heat the area externally to cause the medication to release.

## CHARACTERIZATION OF LIPOSOMES[74]

The behaviour of the liposomes, both in physical and biological system, to a great extent depends upon various factors such as size, shape, lamellarity, entrapment volume etc (Table1). Therefore, liposomes are characterized for these parameters to determine they're in-vivo behaviour to certain extent.

| CHARCTERIZATION PARAMETERS | | ANALYTICAL METHODS/INSTRUMENTATION |
|---|---|---|
| **Chemical Characterization** | | |
| Concentration | Phospholipid | Barlett/Stewart assay, HPLC |
| | Cholesterol | Cholesterol oxidase assay,HPLC |
| | Drug | Method as in individual monograph |
| Phospholipid | Peroxidation | UV absorbance,TBA,iodometric,GLC |
| | Hydrolysis | HPLC,TLC, Fatty Acid Conc. |
| Cholesterol auto-oxidation | | HPLC,TLC, |
| Ant-oxidant degradation | | HPLC,TLC, |
| pH | | pH meter |
| Osmolarity | | Osmometer |
| **Physical Characterization** | | |
| Vesicle | Size & Surface morphology | TEM, Freeze fracture electron microscopy |
| | Size distribution | DLS,Zetasizer,TEM,PCR,gel permeation,exclusion |
| Surface charge | | Free flow electrophoresis |
| Electric surface potential &pH | | Zeta potential measurement, pH probes |
| Lamellarity | | SAXS,$^{31}$NMR, Freeze fracture EM |
| Phase behavior | | Freeze fracture EM,DSC |
| % Entrapment Efficiency | | Minicolumn centrifugation, gel exclusion, ion exachange,protamine aggregation,radiolabelling |
| Drug release | | Diffusion |
| **Biological Characterization** | | |
| Sterility | | Aerobic or anaerobic cultures |
| Pyrogenicity | | LAL test |
| Animal toxicity | | Monitoring survival rates, Histopathology |

**Table 2: Liposome Characterization**

## APPLICATIONS[74-79]

➢ **Common Applications:**

- Cancer chemotherapy
- Gene therapy
- Liposomes as carriers for vaccines
- Liposomes as carrier of drug in oral treatment
- Liposomes for topical applications
- Liposomes for pulmonary delivery
- Against Leishmaniasis
- Lysosomal storage disease
- Cell biological application
- Metal storage disease
- Ophthalmic delivery of drugs

➢ **Applications in Medicine and Pharmacology**

- Liposomes are used in medicine and pharmacology for two main purposes: first, they can be used as a tool, model, or reagent in basic studies of cell interactions, recognition processes, and the mode of action of specific substances. Secondly, liposomes can be used for diagnostic and therapeutic purposes containing different markers or drugs.

- Novel applications for the transport of biotechnology products, including as cloned genes, recombinant proteins, and antisense oligonucleotides, are being made possible by advancements in liposome design.
- Liposomal formulations of all-trans retinoic acid and daunorubicin, which has been approved by the Food and Drug Administration as a first-line therapy for advanced Kaposi's sarcoma associated with AIDS, are two examples of recent advancements. Amphotericin B, doxorubicin, and vincristine are notable examples.
- Seven categories (Table 2) may be used to describe the advantages of drug load in liposomes, which can be administered as (colloidal) solution, aerosol, or in (semi) solid forms, such creams and gels:

| Benefits of drug load in liposome | Examples |
|---|---|
| 1. Improved solubility of lipophilic and amphiphilic drugs | Amphotericin B, porphyrins, minoxidil, some peptides, and anthracyclines, respectively; hydrophilic drugs, such as anticancer agent doxorubicin or acyclovir |
| 2. Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system | Antimonials, amphotericin B, porphyrins, vaccines, immunomodulators |
| 3. Sustained release system of systemically or locally administered liposomes | Doxorubicin, cytosine arabinoside, cortisones, biological proteins or peptides such as vasopressin |
| 4. Site-avoidance mechanism | Doxorubicin andamphotericin B |
| 5. Site-specific targeting | Anti-inflammatory drugs, anti-cancer, anti-infection |
| 6. Improved transfer of hydrophilic, charged molecules | Antibiotics, chelators, plasmids, and genes |
| 7. Improved penetration into tissues | Corticosteroids, anesthetics, and insulin |

**Table 3: Benefits of drug load in liposomes**

## LIPOSOMES IN PARASITIC DISEASES AND INFECTIONS[80-82]

Conventional liposomes are perfect for delivering drug molecules to these macrophages as they are readily ingested by phagocytic cells in the body following intravenous administration. The most well-known examples of this "Trojan horse-like" process are a number of parasite illnesses that often affect MPS cells. They include several fungal diseases and leishmaniasis. In tropical climates, leishmaniasis is a parasite infection of macrophages that affects over 100 million individuals and is frequently fatal. Drugs, primarily antimonials, have effective doses that are not much lower than their poisonous counterparts. Since liposomes gather in the infected cell population, the perfect drug delivery vehicle was postulated. These formulations are derived from the highly productive and effective field of liposome formulations in antifungal medication, primarily using ionosphere amphotericin B.

Liposomes used as carriers for amphotericin B in antifungal medicines have perhaps produced the finest outcomes in human therapy to date. This is the recommended medication for widespread fungal infections, which commonly coexist with chemotherapy, the immune system, or AIDS and are often deadly. Unfortunately, the medicine's ionosphere and neurotoxicity restrict its dose, and the substance itself is quite poisonous. These toxicities typically have something to do with how big the drug molecule or its complex is. It goes without saying that liposome encapsulation drastically lowers toxicity and prevents drug buildup in these organs.

Antiviral and antibacterial treatments can be developed using similar techniques. However, the majority of antibiotics are taken orally; liposome encapsulation should only be taken into consideration when an antibiotic is extremely strong and toxic and must be given parenterally. Due to the interactions of these compounds with bilayers and the high densities of their aqueous solutions, which frequently cause liposomes to float as a creamy layer on the top of the tube, the synthesis of antibiotic-loaded liposomes at substantially high drug-to-lipid ratios may be difficult. Numerous alternative methods, such as topical or pulmonary (by inhalation) delivery, are also being explored. Antivirals encapsulated in liposomes, such as acyclovir, azidothymidine, and ribavirin, have also been demonstrated to lessen toxicity; at present, more thorough studies are being conducted to determine their effectiveness.

## APPLICATIONS OF LIPOSOMES IN CANCER THERAPY[81,83-92]

Cancer treatment has had results with liposome usage. Although it has been thoroughly examined and merits

a thorough evaluation, the use of liposomes in the realm of cancer therapies is outside the purview of this review. Nonetheless, the most effective uses of liposomes in cancer treatments are covered in this article. It has been demonstrated that a variety of liposomal formulations of anti-cancer drugs can deliver the medication to the location of solid tumours with less toxicity than free medicine. Currently, there are many products in the market and in clinical development for use as anti-cancer drug delivery vehicle. The first liposomal medicine authorized by the FDA for the treatment of AIDS patients' Kaposi's sarcoma is Doxil, a PEGylated liposomal formulation. Johnson & Johnson sells the PEGylated liposomal formulation known as Doxil (US) or Calyx (outside-US) that contains the anticancer medication doxorubicin. A discrepancy between the supply and demand for Doxil was noted in 2011 when the production facility was briefly closed as a result of quality control problems. The FDA approved the temporary importation of LipoDox to help with the Doxil shortage in the United States. The FDA authorized the first generic version of Doxil in 2013, and Sun Pharma manufactures LipoDox, which is the same liposomal composition as Doxil in the USA and is created in India.

Doxil was later licensed by the FDA to treat recurrent ovarian cancer after it was shown in a study to be effective against refractory ovarian cancer. It has recently received approval for the treatment of breast cancer in the United States (Barenholz 2012), as well as for the treatment of multiple myeloma in Europe and Canada when combined with decade. Galen's registered brand DaunoXome is the liposomal version of daunorubicin that has FDA approval for treating AIDS-related Kaposi's sarcoma. Myocet, a registered trademark of Cephalon, is a liposomal version of doxorubicin that is not PEGylated. While Myocet and cyclophosphamide were approved for use in Europe to treat metastatic breast cancer, the FDA had not yet given the drug's approval for use in the United States.

Lipoplatin, a liposomal version of cisplatin created by Regulon Inc., is presently being assessed for patients with non-small cell lung cancer in a phase III clinical study. Stimuvax is a liposomal formulation that Oncothyreon developed as an anti-MUC1 cancer vaccine to treat non-small cell lung cancer. It is now undergoing a phase III clinical development. Phase III clinical trials are being conducted for Thermo DOX (Celsion), a thermosensitive liposomal formulation of doxorubicin, to treat patients with primary hepatocellular carcinoma. Phase II trials are being conducted for patients with resistant breast cancer of the chest wall with colon liver metastases.

It has been demonstrated that some liposome formulations of several anticancer drugs are less hazardous than the medication in its free form. Anthracyclines are medications that primarily destroy quickly dividing cells by intercalating into DNA to limit the development of dividing cells. This family of drugs is particularly harmful since these cells may also be found in blood cells, gastrointestinal mucosa, hair, and cancers. Adriamycin, a brand name for doxorubicin HCl manufactured by Ben Venue Laboratories in Bedford, Ohio, is the most widely used and researched. Its rising cardiac toxicity limits its dose in addition to the previously listed acute toxicities. The toxicity was typically lowered to around 50%.

Applications in humans generally shown improved administration tolerability and decreased toxicity, but not very enticing effectiveness. Various formulations are undergoing varying stages of clinical trials, exhibiting inconsistent outcomes.

## LIPOSOMAL DELIVERY: FUTURE CHALLENGES[93]

Despite liposomes demonstrated potential as medication delivery systems, relatively few drugs have reached the commercial manufacturing level. Daunoxome, Ambisome, Doxil, Epaxel, and others are a few to mention. The reticulo-endothelial system's absorption of liposomal delivery systems, their large-scale synthesis, and the phospholipids' instability,

which hinders their commercial development, are the three main issues that these systems face.

**Uptake by Reticulo-endothelial system:**

Liposomes can be made into a solution, an aerosol, a semisolid such as a cream, gel, or a dry powder, and these can be delivered for drug delivery. Liposomes are usually identified as foreign particles after systemic injection, which appears to be the most promising approach for these carrier systems. As a result, they are endocytosed by cells of the mononuclear phagocyte system (MPS), primarily fixed Kupffer cells in the liver and spleen. While this destiny is highly helpful for drug delivery to these cells, it often precludes additional uses, such as site-specific drug delivery by the use of ligands generated on the liposome surface to bind to receptors that are overexpressed on the sick cells. Because of this, researchers began looking for liposomes that would not be quickly absorbed by the MPS. A few numbers of lipid compositions that extended the blood circulation durations of liposomes have been found. In this sense, PEG-coated or sterically stabilized liposomes are excellent examples.

**Large scale production:**

The process of making liposomes includes a number of processes, including sonication, thin-lipid film production, and solvent system evaporation at low pressure. Especially the thin-film preparation, these stages are challenging to complete on a wide scale. Therefore, it is challenging to increase liposome production from a laboratory setting to a large-scale manufacturing setting. According to regulatory standards, it is also not advised to add organic solvents like methanol, chloroform, or other substances at such high concentrations for the purpose of solubilizing and mixing lipids.

**Stability:**

While the usage of liposomes in isolation can help stabilize unstable medications such as tretinoin, the phospholipids utilized in their synthesis are very susceptible to hydrolysis and/or oxidation. Lipid-based goods therefore cannot be kept in storage for an extended amount of time. Occasionally, nevertheless, the products are offered in lyophilized form, which requires reconstitution before usage. Liposomes exhibit chemical instability in addition to physical instability. Furthermore, it has been found that liposomes cannot be sufficiently stabilized by electrostatic stabilization when disintegration substances, such as the proteins and enzymes used in in-vivo applications, are present.

## FUTURE PERSPECTIVES AND CONCLUDING REMARKS[94]

We examined the quantity of articles in Scopus between 1970 and 2020 that had the TITLE-ABS-KEY "liposome," "(liposome AND medicine) or (liposome AND drug)," "(nano AND liposomes AND medicine) or (nano AND liposomes AND drug)," and "(nano AND medicine) or (nano AND drug)." Fascinating findings were discovered.

Fig 11 shows that:

(1) the use of liposomes as drug carriers and in other industries (such as food and cosmetics) began earlier than the use of nanomedicines, i.e., in 1970 as opposed to 1990.

(2) Over time, the percentage of liposomal publications that use liposomes as drug carriers has increased: from 50% in 2000 to 70% in 2010, and 74% in 2020.

(3) Despite the fact that the field of nanomedicine was developed later than the use of liposomes, the number of publications in this field has increased exponentially over time.

(4) There are 3024 papers regarding liposome medicine in 2020, but the extremely low percentages (7%) of medicine/drug nanoliposomes in total nanomedicine/drug detected may be erroneous data.

We hypothesize that compared to the names of nanoparticles, nanocrystals, or nanosuspensions, the combination of the words "nano" and "liposome" may be less commonly used. Up to February 18, 2016, the

FDA indicated that over 500 applications for liposomes had been submitted. Of these applications, 3% were NDAs, 1% were ANDAs, and 96% were INDs. About 100 submissions were made for combination treatment, which involves using liposomes in addition to another medicine. Many liposomal products will soon go from the laboratory bench to the pilot plant and market, according to data gathered from the pharmaceutical industry and laboratory level.

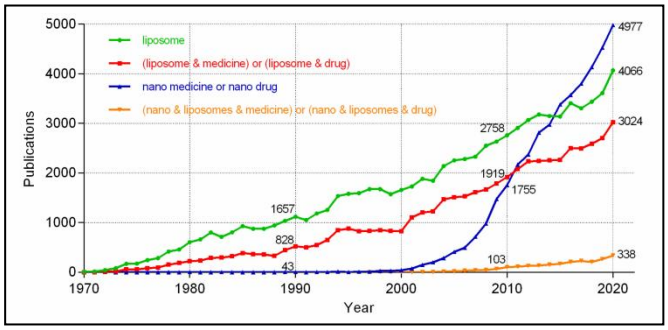



**Fig 11**: The comparison profiles of articles in the Scopus year range between 1970 and 2020 with TITLE-ABS-KEY set to "liposome", "(liposome AND medicine) or (liposome AND drug)", "(nano AND liposomes AND medicine) or (nano AND liposomes AND drug)," and "(nano AND medicine) or (nano AND drug)".

Over the course of two decades, liposome procedures have been refined since the approval of Doxil, the first liposome product, in 1995. Based on the large number of publications and commercial goods, we provide a summary of the successful experience and pain spots. Depending on the demands and requirements of humans, liposomes can be well-designed and perform their intended roles. The accelerated blood clearance (ABC) phenomenon of PEGylated liposomes, individual variations in the EPR effect, scale-up issues, reproducibility/consistency across batches and manufacturing sites, and excipient control are some of the significant challenges encountered during development and commercialized production.

There has been much debate lately over "where are we in the development path of nanomedicines?" We have an optimistic outlook when we reflect on the past about the performance and application scenario of liposomes. The Chinese NMPA has authorized three different types of liposomal products: Amphotericin Bliposome, Doxorubicin Hydrochloride Liposome, and Lipusu (paclitaxel liposome). Furthermore, China is home to both major pharmaceutical corporations and upstart, creative small businesses that are creating nanomedicines such as polymeric micelles, liposomes, nanocrystals, and inorganic particles. Simultaneously, the NMPA has designated the subject of "safety and quality evaluation of nanomedicine" as a major project for the advancement of regulatory science in 2019. In response to the seamless integration of nanomedicines in the future, we are putting together a regulatory framework.

## II. CONCLUSION

Applications for liposomes in medicine are numerous and varied. As intracellular delivery vehicles for anti-sense compounds, ribosomes, proteins/peptides, and DNA, liposomes are particularly promising. Liposomes that possess extended circulation residence durations, which improve medication delivery to disease sites, are already gaining clinical acceptability. Additionally, liposomes facilitate the targeting of specific sick cells inside the location of the illness. It has been discovered that liposomes are incredibly helpful medication delivery vehicles. Regardless of the solubility characteristics of the drug material, the flexibility of their behaviour may be used to administer the medication by any route of administration. Future advancements in the delivery of medications and genes via liposomes are expected given their promising nature.

## III. REFERENCES

[1] FDA U. Liposome Drug Products: Chemistry, Manufacturing, and Controls; Human Pharmacokinetics and Bioavailability; and Labelling Documentation. Guid. Ind. 2018 Apr.

[2] Mazur F, Bally M, Städler B, Chandrawati R. Liposomes and lipid bilayers in biosensors. Advances in colloid and interface science. 2017 Nov 1;249:88-99.

[3] Düzgüneş N, Gregoriadis G. Introduction: the origins of liposomes: Alec Bangham at Babraham. In Methods in enzymology 2005 Jan 1 (Vol. 391, pp. 1-3). Academic Press.

[4] Bangham AD, Horne RW. Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope. Journal of molecular biology. 1964 Jan 1;8(5):660-IN10.

[5] Sahoo SK, Labhasetwar V. Nanotech approaches to drug delivery and imaging. Drug discovery today. 2003 Dec 15;8(24):1112-20.

[6] Gabizon A, Goren D, Cohen R, Barenholz Y. Development of liposomal anthracyclines: from basics to clinical applications. Journal of controlled release. 1998 Apr 30;53(1-3):275-9.

[7] Allen TM. Liposomes: opportunities in drug delivery. Drugs. 1997 Oct;54:8-14.

[8] Zhang M, Gao S, Yang D, Fang Y, Lin X, Jin X, Liu Y, Liu X, Su K, Shi K. Influencing factors and strategies of enhancing nanoparticles into tumors in vivo. Acta Pharmaceutica Sinica B. 2021 Aug 1;11(8):2265-85.

[9] Atrooz OM. Effects of alkylresorcinolic lipids obtained from acetonic extract of Jordanian wheat grains on liposome properties. Int J Biol Chem. 2011;5(5):314-21.

[10] Benech RO, Kheadr EE, Laridi R, Lacroix C, Fliss I. Inhibition of Listeria innocua in cheddar cheese by addition of nisin Z in liposomes or by in situ production in mixed culture. Applied and environmental microbiology. 2002 Aug;68(8):3683-90.

[11] Shehata T, Ogawara KI, Higaki K, Kimura T. Prolongation of residence time of liposome by surface-modification with mixture of hydrophilic polymers. International journal of pharmaceutics. 2008 Jul 9;359(1-2):272-9.

[12] Johnston MJ, Semple SC, Klimuk SK, Ansell S, Maurer N, Cullis PR. Characterization of the drug retention and pharmacokinetic properties of liposomal nanoparticles containing dihydrosphingomyelin. Biochimica et Biophysica Acta (BBA)-Biomembranes. 2007 May 1;1768(5):1121-7.

[13] Hofheinz RD, Gnad-Vogt SU, Beyer U, Hochhaus A: Liposomal encapsulated anti-cancer drugs. Anticancer Drugs 2005, 16:691–707.

[14] Omri A, Suntres ZE, Shek PN: Enhanced activity of liposomal polymyxin B against Pseudomonas aeruginosa in a rat model of lung infection. Biochem Pharmacol 2002, 64:1407–1413.

[15] Schiffelers RM, Storm G, Bakker-Woudenberg IA. Host factors influencing the preferential localization of sterically stabilized liposomes in Klebsiella pneumoniae-infected rat lung tissue. Pharmaceutical research. 2001 Jun; 18:780-7.

[16] Stano P, Bufali S, Pisano C, Bucci F, Barbarino M, Santaniello M, Carminati P, Luisi PL: Novel camptothecin analogue (gimatecan)-containing liposomes prepared by the ethanol injection method. J Liposome Res 2004, 14:87–109.

[17] Hemanthkumar M, Spandana V. Liposomal encapsulation technology a novel drug delivery system designed for ayurvedic drug preparation. IRJP. 2011;2(10):4-7.

[18] Fitzmaurice C, Dicker D, Pain A, Hamavid H, Moradi-Lakeh M, MacIntyre MF, Allen C, Hansen G, Woodbrook R, Wolfe C, Hamadeh RR. The global burden of cancer 2013. JAMA oncology. 2015 Jul 1;1(4):505-27.

[19] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians. 2015 Mar;65(2):87-108.

[20] Bhardwaj A, Srivastava SK, Singh S, Arora S, Tyagi N, Andrews J, McClellan S, Carter JE, Singh AP. CXCL12/CXCR4 signaling counteracts docetaxel-induced microtubule stabilization via p21-activated kinase 4-dependent activation of LIM domain kinase 1. Oncotarget. 2014 Nov;5(22):11490.

[21] Tyagi N, Bhardwaj A, Singh AP, McClellan S, Carter JE, Singh S. p-21 activated kinase 4 promotes proliferation and survival of pancreatic cancer cells through AKT-and ERK-dependent activation of NF-κB pathway. Oncotarget. 2014 Sep;5(18):8778.

[22] Srivastava SK, Bhardwaj A, Arora S, Tyagi N, Singh AP, Carter JE, Scammell JG, Fodstad Ø, Singh S. Interleukin-8 is a key mediator of FKBP51-induced melanoma growth, angiogenesis and metastasis. British journal of cancer. 2015 May;112(11):1772-81.

[23] Srivastava SK, Bhardwaj A, Arora S, Tyagi N, Singh S, Andrews J, McClellan S, Wang B, Singh AP. MicroRNA-345 induces apoptosis in pancreatic cancer cells through potentiation of caspase-dependent and-independent pathways. British journal of cancer. 2015 Aug;113(4):660-8.

[24] MacDonald V. Chemotherapy: managing side effects and safe handling. The Canadian Veterinary Journal. 2009 Jun;50(6):665.

[25] Ramirez LY, Huestis SE, Yap TY, Zyzanski S, Drotar D, Kodish E. Potential chemotherapy side effects: what do oncologists tell parents? Pediatric blood & cancer. 2009 Apr;52(4):497-502.

[26] Iwamoto T. Clinical application of drug delivery systems in cancer chemotherapy: review of the efficacy and side effects of approved drugs. Biological and pharmaceutical bulletin. 2013 May 1;36(5):715-8.

[27] Tyagi N, Rathore SS, Ghosh PC. Enhanced killing of human epidermoid carcinoma (KB) cells by treatment with ricin encapsulated into sterically stabilized liposomes in combination with monensin. Drug Delivery. 2011 Aug 1;18(6):394-404.

[28] Tyagi N, Rathore SS, Ghosh PC. Efficacy of liposomal monensin on the enhancement of the antitumour activity of liposomal ricin in human epidermoid carcinoma (KB) cells. Indian Journal of Pharmaceutical Sciences. 2013 Jan;75(1):16.

[29] Arora S, Tyagi N, Bhardwaj A, Rusu L, Palanki R, Vig K, Singh SR, Singh AP, Palanki S, Miller ME, Carter JE. Silver nanoparticles protect human keratinocytes against UVB radiation-induced DNA damage and apoptosis: potential for prevention of skin carcinogenesis. Nanomedicine: Nanotechnology, Biology and Medicine. 2015 Jul 1;11(5):1265-75.

[30] Alving CR. Macrophages as targets for delivery of liposome-encapsulated antimicrobial agents. Advanced Drug Delivery Reviews. 1988 Oct 1;2(1):107-28.

[31] Allison AC, Gregoriadis G. Liposomes as immunological adjuvants. Nature. 1974 Nov 15;252(5480):252-.

[32] Deamer D, Uster P. Liposome preparation methods and monitoring liposome fusion. Introduction of Macromolecules into viable Mammalian Cells, Alan R. Liss, New York. 1980:205.

[33] De Marie S, Jankengt R, Bakker-Woudenberg IA. Clinical use of liposomal and lipid-complexed amphotericin B. Journal of Antimicrobial Chemotherapy. 1994 May 1;33(5):907-16.

[34] Lasic DD, Papahadjopoulos D. Liposomes revisited. Science. 1995 Mar 3;267(5202):1275-6.

[35] Emanuel N, Kedar E, Bolotin EM, Smorodinsky NI, Barenholz Y. Preparation and characterization of doxorubicin-loaded sterically stabilized immunoliposomes. Pharmaceutical research. 1996 Mar; 13:352-9.

[36] Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery. Chemical reviews. 2015 Oct 14;115(19):10938-66.

[37] Kim T, Kim J, Kim S. Extended-release formulation of morphine for subcutaneous administration. Cancer chemotherapy and pharmacology. 1993 May; 33:187-90.

[38] Fan Y, Marioli M, Zhang K. Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. Journal of pharmaceutical and biomedical analysis. 2021 Jan 5; 192:113642.

[39] Wang N, Wu T, Wang T. Liposomes used as a vaccine adjuvant-delivery system. InLiposomes 2017 Oct 25. IntechOpen.

[40] Barenholz YC. Doxil®—The first FDA-approved nano-drug: Lessons learned. Journal of controlled release. 2012 Jun 10;160(2):117-34.

[41] Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, Deng Y. A review on phospholipids and their main applications in drug delivery systems. Asian journal of pharmaceutical sciences. 2015 Apr 1;10(2):81-98.

[42] Laouini A, Jaafar-Maalej C, Limayem-Blouza I, Sfar S, Charcosset C, Fessi H. Preparation, characterization and applications of liposomes: state of the art. Journal of colloid Science and Biotechnology. 2012 Dec 1;1(2):147-68.

[43] Marqués-Gallego P, de Kroon AI. Ligation strategies for targeting liposomal nanocarriers. BioMed research international. 2014 Jul 14;2014.

[44] Farge E, Devaux PF. Shape changes of giant liposomes induced by an asymmetric transmembrane distribution of phospholipids. Biophysical journal. 1992 Feb 1;61(2):347-57.

[45] Bitounis D, Fanciullino R, Iliadis A, Ciccolini J. Optimizing druggability through liposomal formulations: new approaches to an old concept. International Scholarly Research Notices. 2012;2012.

[46] Ellens H, Bentz J, Szoka FC. Destabilization of phosphatidylethanolamine liposomes at the hexagonal phase transition temperature. Biochemistry. 1986 Jan 1;25(2):285-94.

[47] Magarkar A, Dhawan V, Kallinteri P, Viitala T, Elmowafy M, Róg T, Bunker A. Cholesterol level affects surface charge of lipid membranes in saline solution. Scientific reports. 2014 May 21;4(1):5005.

[48] Kirby C, Clarke J, Gregoriadis G. Effect of the cholesterol content of small unilamellar liposomes on their stability in vivo and in vitro. Biochemical Journal. 1980 Feb 15;186(2):591-8.

[49] Lasic DD. On the thermodynamic stability of liposomes. Journal of colloid and interface science. 1990 Nov 1;140(1):302-4.

[50] Mayer LD, Hope MJ, Cullis PR, Janoff AS. Solute distributions and trapping efficiencies observed in freeze-thawed multilamellar vesicles. Biochimica et Biophysica Acta (BBA)-Biomembranes. 1985 Jul 11;817(1):193-6.

[51] Vyas SP, Khar RK. Targeted & controlled drug delivery: novel carrier systems. CBS publishers & distributors; 2004.

[52] Deamer D, Bangham AD. Large volume liposomes by an ether vaporization method. Biochimica et Biophysica Acta (BBA)-Nucleic Acids and Protein Synthesis. 1976 Sep 7;443(3):629-34.

[53] Schieren H, Rudolph S, Finkelstein M, Coleman P, Weissmann G. Comparison of large unilamellar vesicles prepared by a petroleum ether vaporization method with

multilamellar vesicles: ESR, diffusion and entrapment analyses. Biochimica et Biophysica Acta (BBA)-General Subjects. 1978 Aug 3;542(1):137-53.

[54] Himanshu A, Sitasharan P, Singhai AK. Liposomes as drug carriers. IJPLS. 2011;2(7):945-51.

[55] Kataria S, Sandhu P, Bilandi AJ, Akanksha M, Kapoor B. Stealth liposomes: a review. International journal of research in ayurveda & pharmacy. 2011 Sep 1;2(5).

[56] Shaheen SM, Shakil Ahmed FR, Hossen MN, Ahmed M, Amran MS, Ul-Islam MA. Liposome as a carrier for advanced drug delivery. Pak J Biol Sci. 2006;9(6):1181-91.

[57] Daemen T, Hofstede G, Ten Kate MT, Bakker-Woudenberg IA, Scherphof GL. Liposomal doxorubicin-induced toxicity: Depletion and impairment of phagocytic activity of liver macrophages. International journal of cancer. 1995 May 29;61(5):716-21.

[58] Kirby CJ, Gregoriadis G. A simple procedure for preparing liposomes capable of high encapsulation efficiency under mild conditions. InLiposome technology 2019 Jul 23 (pp. 19-27). CRC Press.

[59] Alpes H, Allmann K, Plattner H, Reichert J, Rick R, Schulz S. Formation of large unilamellar vesicles using alkyl maltoside detergents. Biochimica et Biophysica Acta (BBA)-Biomembranes. 1986 Nov 17;862(2):294-302.

[60] Sutradhar KB, Amin ML. Nanotechnology in cancer drug delivery and selective targeting. International scholarly research notices. 2014;2014.

[61] Bozzuto G, Molinari A. Liposomes as nanomedical devices. International journal of nanomedicine. 2015 Feb 2:975-99.

[62] Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. Nanoscale research letters. 2013 Dec; 8:1-9.

[63] Haran G, Cohen R, Bar LK, Barenholz Y. Transmembrane ammonium sulfate gradients in liposomes produce efficient and stable entrapment of amphipathic weak bases. Biochimica et Biophysica Acta (BBA)-Biomembranes. 1993 Sep 19;1151(2):201-15.

[64] Lasic DD, Frederik PM, Stuart MC, Barenholz Y, McIntosh TJ. Gelation of liposome interior A novel method for drug encapsulation. FEBS letters. 1992 Nov 9;312(2-3):255-8.

[65] Qiu L, Jing N, Jin Y. Preparation and in vitro evaluation of liposomal chloroquine diphosphate loaded by a transmembrane pH-gradient method. International journal of pharmaceutics. 2008 Sep 1;361(1-2):56-63.

[66] Maurya P. PHARMACEUTICAL LIPOSOMAL DRUG DELIVERY: A COMPLETE REVIEW OF NEW DELIVERY SYSTEM.

[67] Jain NK, editor. Controlled and novel drug delivery. New Delhi: CBS publishers & distributors; 1997.

[68] Kersten GF, Crommelin DJ. Liposomes and ISCOMS as vaccine formulations. Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes. 1995 Jul 17;1241(2):117-38.

[69] Horton K. Disertation for degree of Advanced Studies in Chemical Engineering. Universitat Rovira I Virgili. 2003.

[70] Gosavi AA, Salunkhe KK. Updated Review On: Liposome.

[71] Sharma A, Straubinger RM. Novel taxol formulations: preparation and characterization of taxol-containing liposomes. Pharmaceutical research. 1994 Jun; 11:889-96.

[72] Szoka FC. Liposomal drug delivery: current status and future prospects. Membrane fusion. 2019 Nov 14:845-90.

[73] Vingerhoeds MH, Storm G, Crommelin DJ. Immunoliposomes in vivo. Immunomethods. 1994 Jun 1;4(3):259-72.

[74] Vyas SP, Khar RK. Targeted & controlled drug delivery: novel carrier systems. CBS publishers & distributors; 2004.

[75] Banerjee R, Tyagi P, Li S, Huang L. Anisamide-targeted stealth liposomes: a potent carrier for targeting doxorubicin to human prostate cancer cells. International journal of cancer. 2004 Nov 20;112(4):693-700.

[76] Gill PS, Espina BM, Muggia F, Cabriales S, Tulpule A, Esplin JA, Liebman HA, Forssen E, Ross ME, Levine AM. Phase I/II clinical and pharmacokinetic evaluation of liposomal daunorubicin. Journal of clinical Oncology. 1995 Apr;13(4):996-1003.

[77] Guaglianone P, Chan K, DelaFlor-Weiss E, Hanisch R, Jeffers S, Sharma D, Muggia F. Phase I and pharmacologie study of liposomal daunorubicin (DaunoXome). Investigational new drugs. 1994 Jun; 12:103-10.

[78] Eckardt JR, Campbell E, Bums HA, Weiss GR, Rodriguez GI, Fields SM, Thurman AM, Peacock NW, Cobb P, Rothenberg MA, Ross ME. A phase II trial of DaunoXome, liposome-encapsulated daunorubicin, in patients with metastatic adenocarcinoma of the colon. American journal of clinical oncology. 1994 Dec 1;17(6):498-501.

[79] Schurmann D, Dormann A, Grunewald T, Ruf B. Successful treatment of AIDS-related pulmonary Kaposi's sarcoma with liposomal daunorubicin. European Respiratory Journal. 1994 Apr 1;7(4):824-5.

[80] New RR, Chance ML, Thomas SC, Peters W. Antileishmanial activity of antimonials entrapped in liposomes. Nature. 1978 Mar 2;272(5648):55-6.

[81] Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K. Liposome: classification, preparation, and applications. Nanoscale research letters. 2013 Dec; 8:1-9.

[82] Svenson CE, Popescu MC, Ginsberg RC. Liposome treatments of viral, bact and protozoal infections. Crit Rev Microbiol. 1988;15: S1-31.

[83] Storm G, Roerdink FH. A Steerenberg, P.; De Jong, WH; Crommelin, DJ Influence of lipid composition on the antitumor activity exerted by doxorubicin-containing liposomes in a rat solid tumor model. Cancer Res. 1987; 47:3366-72.

[84] Akbarzadeh A, Mikaeili H, Zarghami N, Mohammad R, Barkhordari A, Davaran S. RETRACTED ARTICLE: Preparation and in vitro evaluation of doxorubicin-loaded Fe3O4 magnetic nanoparticles modified with biocompatible copolymers. International journal of nanomedicine. 2012 Feb 1:511-26.

[85] Akbarzadeh A, Samiei M, Davaran S. Magnetic nanoparticles: preparation, physical properties, and applications in biomedicine. Nanoscale research letters. 2012 Dec; 7:1-3.

[86] Valizadeh A, Mikaeili H, Samiei M, Farkhani SM, Zarghami N, Kouhi M, Akbarzadeh A, Davaran S. Quantum dots: synthesis, bioapplications, and toxicity. Nanoscale research letters. 2012 Dec; 7:1-4.

[87] Akbarzadeh A, Samiei M, Joo SW, Anzaby M, Hanifehpour Y, Nasrabadi HT, Davaran S. RETRACTED ARTICLE: Synthesis, characterization and in vitro studies of doxorubicin-loaded magnetic nanoparticles grafted to smart copolymers on A549 lung cancer cell line. Journal of nanobiotechnology. 2012 Dec;10(1):1-3.

[88] Du S, Deng Y. Studies on the encapsulation of oxymatrine into liposomes by ethanol injection and pH gradient method. Drug development and industrial pharmacy. 2006 Jan 1;32(7):791-7.

[89] Chou H, Lin H, Liu JM. A tale of the two PEGylated liposomal doxorubicins. OncoTargets and therapy. 2015 Jul 13:1719-20.

[90] Berger JL, Smith A, Zorn KK, Sukumvanich P, Olawaiye AB, Kelley J, Krivak TC. Outcomes analysis of an alternative formulation of PEGylated liposomal doxorubicin in recurrent epithelial ovarian carcinoma during the drug shortage era. OncoTargets and therapy. 2014 Aug 8:1409-13.

[91] Fantini M, Gianni L, Santelmo C, Drudi F, Castellani C, Affatato A, Nicolini M, Ravaioli A. Lipoplatin treatment in lung and breast cancer. Chemotherapy research and practice. 2011;2011.

[92] Bradbury PA, Shepherd FA. Immunotherapy for lung cancer. Journal of Thoracic Oncology. 2008 Jun 1;3(6): S164-70.

[93] Lasic DD. Novel applications of liposomes. Trends in biotechnology. 1998 Jul 1;16(7):307-21.

[94] Kapoor M, Lee SL, Tyner KM. Liposomal drug product development and quality: current US experience and perspective. The AAPS journal. 2017 May; 19:632-41.

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