LIPOSOMES: A NOVEL DRUG DELIVERY SYSTEM

Abstract:

The liposome, a new drug delivery system, is a lipid vesicle made of a phospholipid membrane that has significant advantages over conventional dosage forms for the targeted and site-specific action of hydrophilic and lipophilic drugs because the drug is encapsulated in the core, protecting it from microbial or enzymatic degradation. Depending on the nature of the liposome and the medicine as well as how they interact with one another, different preparation techniques are employed to create liposomes. Before utilizing formulation, a number of characteristics, including chemical, physical, and biological characterization, are evaluated again to make sure the intended properties of the final product are present. stabilization is also done to check the stability of the product before its use and after reaching its site of action, liposomal formulation can be useful for anticancer drugs like doxorubicin, mitoxantrone, cisplatin, docetaxel, as in anti-infectives like penicillin vancomycin rifampicin, as for ocular drugs like tombolo, acetazolamide, orzo amide also for vaccine adjuvant scan antiviral so many more. The research conducted up to this point in examining the period and current advancements in the liposomal drug delivery method have benefited from this review.

Keywords: Depending on the nature of the liposome and the medicine as well as how they interact with one another, different preparation techniques are employed to create liposomes.

Author

Dr. Nidhi N. Chauhan
Department of Pharmaceutics
Laxminarayandev College of Pharmacy
Bharuch, Gujarat. India.
nidhi.berawala11687@gmail.com

Dr. Vaishali Patel
Department of Pharmaceutics
Laxminarayandev College of Pharmacy
Bharuch, Gujarat. India
laxminarayandevcollege@gmail.com.

Anand Chavda
Department of Pharmaceutics
Laxminarayandev College of Pharmacy
Bharuch, Gujarat. India
laxminarayandevcollege@gmail.com

Anil Chavda
Department of Pharmaceutics
Laxminarayandev College of Pharmacy
Bharuch, Gujarat. India
laxminarayandevcollege@gmail.com

Kashish Joshi
Department of Pharmaceutics
Laxminarayandev College of Pharmacy
Bharuch, Gujarat. India
laxminarayandevcollege@gmail.com

I. INTRODUCTION

Vesicular drug delivery systems are designed to provide pharmaceuticals with lower toxicity and controlled release, in contrast to conventional dosage forms. The spherical colloid vesicles known as liposomes are made of cholesterol, a non-toxic surfactant, phospholipids, sphingolipids, glycolipids, and an aqueous phase that contains active medicines that release at a set rate, such as hormones, peptides, enzymes, etc. Numerous liposome characteristics, such as size, charge, lamellarity, composition, and application, can be generated. Liposomes were created more than 55 years ago by British haematologist Dr. Alec D. Bingham. They are flexible instruments used in many fields, such as biology, biochemistry, and medicine. Several different compounds were transported into aqueous compartments in the 1960s using liposomes. For the first time, a replacement treatment was delivered via a liposome in the 1970s.

Following are some modes of action by which liposomes can act within the body: -
- After the liposome and cell membrane have merged, the liposome's contents are released into the cell.
- Liposomes can penetrate cells, integrate their phospholipids into the cell membrane, and release the medication.
- If phagocytic cells are present when the liposome is picked up, the drug is released when the lysosomes' original phospholipid walls are applied to them.

II. ADVANTAGES AND DISADVANTAGES

Fewer dosages are required for medications that are non-toxic, non-immunogenic, biocompatible, biodegradable, and released at a predefined rate, which reduces side effects and increases patient compliance. advantageous for medications that the body breaks down more quickly. Targeted and site-specific pharmacological administration is necessary for the treatment of cancer. compatible with pharmaceuticals that are hydrophilic, lipophilic, and amphiphilic. The medication's internal aqueous phase encapsulation and seclusion from the immediate external environment contribute to greater drug stability.

Costly because specialized equipment and techniques are required. Drug therapy might occasionally fail due to dosage dumping and leakage.

III. CLASSIFICATION OF LIPOSOMES

Liposomes are categorized depending on
- Based on its molecular structure
- Based on the formulation process
- Composition and its use
- Based on structural parameters
- Conventional liposome
- Special liposome

1. Based on its Molecular Structure

Table 1: Based on its Molecular Structure

Vesicle type	Abbreviation	Diameter	No. of the lipid bilayer
Unilamellar vesicle	UV	All size	1
Small unilamellar vesicle	SUV	20-100 nm	1
Medium unilamellar vesicle	MUV	>100 nm	1
Large unilamellar vesicle	LUV	>100 nm	1
Giant unilamellar vesicle	GUV	>1 um	1
Oligolamellar vesicle	OLV	0.1 – 1 um	Approx. 0.5
Multi lamellar vesicle	MLV	>0.5 um	5-25
Multi vesicular vesicle	MV	>1 um	Multi structure

2. Based on the Formulation Process:

Table 2: Based on the Formulation Process

Formulation Process	Vesicle type
Reverse /oligomer vesicle made by a reverse phase evaporation method	REV
Multi lamellar vesicle made by a reverse phase evaporation method	MLV – REV
Stable pluri lamellar vesicle	SPL
Frozen and thawed multi lamellar	FAT – MLV
Vesicle prepared by extrusion technique	VET
Dehydration rehydration method	DRV

3. Based on Structural Parameters:

Table 3: Based on Structural Parameters

Types of Liposomes Based on Structural Parameters	Size (micrometer)
Multilamellar liposome (MLV)	0.1-0.5 micrometer
Unilamellar liposomes	
Small unilamellar liposomes (SUV)	0.02-0.05 micrometer
Large unilamellar liposomes (LUV)	More than 0.06 micrometers
Multivesicular liposomes (MVV)	2-40 micrometre
Oligolamellar liposomes	0.1-10 micrometer in size
GL	10-1000 micrometer

4. **Based on Conventional Liposomes:**

- Normalise the mixture of natural lecithin
- Glycolipid-loaded liposome
- Synthetic phospholipid with the same chain as natural phospholipid

5. **Special Liposomes:**

- Carbohydrate coated
- Bipolar fatty acid
- Lipoprotein coated
- Methyl–methylene linked
- Multiple encapsulated
- Antibody directed

IV. COMPOSITION OF LIPOSOMES

1. **Phospholipids:** More than half of the weight of the biological membrane of a liposome is made up of the most frequently used component, glyceryl-containing phospholipid. The molecule's main structural component is glycerol's phosphoric ester. Saturated fatty acids can be used to make a stable emulsion if unsaturated fatty acids are not available. The following are some phospholipid examples:

- PC phospholipid choline
- Ethanolamine phosphonate PE
- Phosphodiester serine PS
- PI phosphorylated inositol

2. **Sphingolipids**: This important element exhibits three properties in both plant and animal cells.A molecule of fatty acidsAn element of sphingosineSphingomyelin and glycosphingolipid are the two sphingolipid kinds that are employed by a head group the most common, and they can range from basic alcohol like choline to very complex carbohydrates

3. **Cholesterol:** Because of the liposome's low transition temperature, which causes issues with cell membrane packaging and drug leakage, cholesterol is added to the phospholipid in a ratio of 1:1 or 2:1 to assist address these concerns.

   The cell membrane is wrappedReduce the permeability and solubility of the hydrophilic medication.By stabilizing the liposome against plasma proteins like albumin, macroglobulin, and transferrin, you can prevent it from becoming unstable.

4. **Synthetic Phospholipid:**
   For saturated phospholipid: DPPE, DPPS, DPPA, DPPG
   In regards to unsaturated phospholipid: DOPC, DOPG
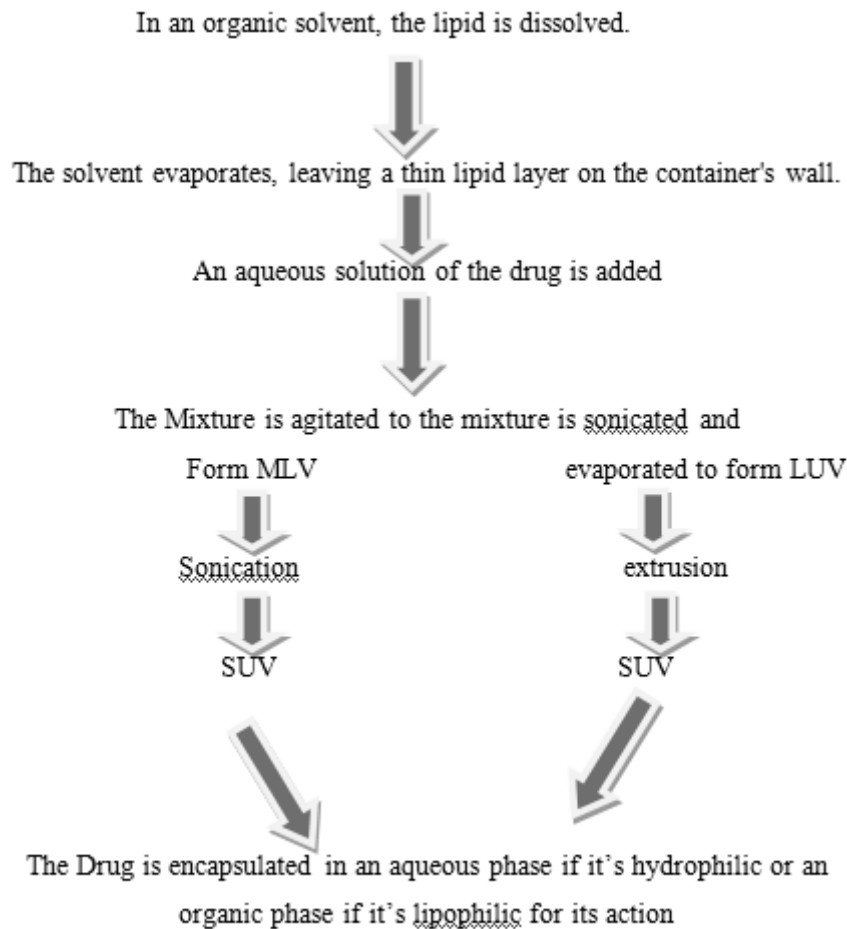
V. THE GENERAL METHOD OF PREPARATION

In an organic solvent, the lipid is dissolved.

↓

The solvent evaporates, leaving a thin lipid layer on the container's wall.

↓

An aqueous solution of the drug is added

↓

The Mixture is agitated to the mixture is sonicated and
Form MLV   evaporated to form LUV

Sonication ↓   extrusion ↓

SUV   SUV

The Drug is encapsulated in an aqueous phase if it's hydrophilic or an organic phase if it's lipophilic for its action

Figure 1

1. **Physical Dispersion:**
   ● lipid film hydration method (handshaking and non-shaking)
   ● freeze drying
   ● micro emulsification
   ● sonication
   ● membrane extrusion
   ● freeze-thaw sonication

2. **Mechanical Dispersion:**
   ● ltrasonic method

3. **Solvent Dispersion:**
   ● ethanol injection
   ● ether injection
   ● reverse phase evaporation

4. **Detergent Solubilisation:**
   - Size transformation
   - freeze-thaw extrusion
   - dehydration rehydration

VI. ACTIVE LOADING

1. **Lyophilisation:** Before they reach their destination, natural extracts usually degrade as a result of oxidation and other chemical processes. A common method utilised in the production of many medicinal goods is freeze-drying. These products are typically lyophilized from simple aqueous solutions. There are several instances where pharmaceutical products are produced using a process that involves freeze-drying from organic co-solvent systems, even though normally, water is the only solvent that needs to be removed from the solution using the freeze-drying method. Freeze-drying, also known as lyophilisation, is the method of removing water from frozen items by using very low pressures. The materials that this method is typically used to dry would be harmed by heat-drying due to their thermos stability. Liposomal technology, which has a lot of potential in this area, can be used to address concerns with long-term stability. Encapsulated components may leak during freeze-drying and reconstitution, according to studies. According to recent studies, liposomes can preserve up to 100% of their initial contents (a carbohydrate that is typically present in large amounts in animals) after being freeze-dried with sufficient treehouse. It demonstrates how effective treehouse is as a liposome cry (freeze) protectant. A freeze-drier can be as big as an industrial machine or as little as a laboratory model.

2. **Sizing:** A liposome's size properties greatly influence what applications it can be utilized for. The therapeutic use of liposomes is based on the physical stability and integrity of lipid bilayers; as a result, the liposome generation process must be predictable and repeatable with particle size distribution within a specific size range. Lipid-based formulations can be created as site-specific drug delivery vehicles that are:

   - Mostly cleared by macrophages and Kuepfer cells in the liver.
   - Transport liposome-incorporated material to target tissue,and organ, efficiently while avoiding detection of the active substance by the reticuloendothelial system. Liposomes are typically sized by sequential extrusion through a polycarbonate membrane (PCM) at relatively low pressure. Both LUV and MLV can be processed using the membrane extrusion technique to create LUVETs. The membrane has pores that are 0.27 micrometres in size. The alternative method for sizing liposomes is gel chromatography, which is mostly employed to separate encapsulated components from the liposomes.

   The third technique for sizing liposomes is sonication, although it has the following drawbacks:
   - It is challenging to exclude oxygen, which causes an oxidation reaction.
   - Metal particles shed by the titanium probe caused pollution.
   - They are ineligible for use with some agents because they can produce aerosols.

The aforementioned issues are primarily related to probe sonication, although bath sonication can solve them.

VII. PASSIVE LOADING TECHNIQUE

1. Physical Dispersion

- **Lipid Film Method:** It is the first and simplest method for making liposomes that Bingham discovered; in it, the lipid film is hydrated using an organic solvent, which is then evaporated using an evaporator while under vacuum, followed by the addition of an aqueous buffer to create a liposome.
  One disadvantage of this technology is the low interior volume. To improve encapsulation effectiveness, diethyl ether is utilized.

- **Handshaking Method:** A 250 ml RBF is filled with a 2:1 mixture of lipid and organic solvent (chloroform and methanol), which is the simplest and most often used approach. The organic solvent is then burned out at 30 c, leaving behind a dry, solid lipid residue that may be extracted with the help of a rotatory evaporator that is coupled to a vacuum pump operating at 60 rpm. The flask is now detached from the evaporator, and after a second nitrogen flush, 5 ml of phosphate buffer is adding the milky white suspension has formed, the flask is reattached to the evaporator and spun for 30 minutes at 60 rpmrmed. After that, the suspension is left to stand for an additional two hours to complete the swelling process.

- **Non-Shaking Method:** It is similar to the shaking strategy, with the exception that focus is paid to the swelling process. The conical flask is coated with the lipid solution in the chloroform and methanol mixture, and the solution is allowed to evaporate at room temperature by passing nitrogen through the flask until the opacity of the dry film disappears. The addition of bulk liquid causes the lipid to swell after it has been hydrated. A 10 to 20 ml addition of 0.2 M sucrose in distilled water is then made while tilting the flask to one side. The flask is then allowed to let the solution gently run over the lipid layer at the bottom before slowly returning to its upright position. The flask is shut off and flushed with nitrogen.

  ➢ **Freeze Drying:** Another method of dispersing the lipid in a completely split condition before adding aqueous media is by freeze-drying the lipid after it has been dissolved in an appropriate organic solvent. The organic solvent used the most usually is tertiary butanol.

  ➢ **Micro Emulsification of Liposomes:** A tool known as a micro fluidizer is used to create small vesicles from concentrated lipid suspension. The fluidizer can receive the lipid in the form of a large MLV suspension. This device pumps the fluid at a very high pressure past a 5-micron screen. Then, two streams of fluid collide at a right angle and with considerable speed as a result of forced, long microchannel. The collected fluid can be cycled through the pump and interaction chamber until sphere-shaped vesicles are produced.

- ➢ **Sonication:** The lipid solution is supplied energy and the size of the vesicles is decreased by utilizing this method. To do this, the MLV can be ultrasonically irrigated before the explosion. There are two methods of sonication: While method B employs a probe solicitor, Method A employs a bath solicitor. Bath solicitors are utilized for huge amounts of diluted lipids. A probe solicitor is used for suspensions that require high energy in tiny amounts. Probe solicitors have the disadvantage that the preparation may become contaminated with metal from the probe's tip. Using this method, tiny unilamellar vesicles are created, which are then purified using ultracentrifugation.

- ➢ **Membrane Extrusion of Liposomes:** This method reduces the size by passing them through a membrane filter with a predetermined pore size. The two types of membrane filters are the tortuous type and the nucleation track type. For sterile filtering, the former is used in this haphazard path that develops between the crisscrossing fibres. The average diameter of these fibers is controlled by the density of the fibres in the matrix. Liposomes larger than the channel diameter become caught when one attempts to transfer them across such a membrane. The thin, continuous sheet of carbonates that make up the nucleation track is applied to the surface. Liposomes used to process LUV and MLV, feature straight-sided, precise-diameter pore pores, which means they will provide less resistance.

- ➢ **Freeze-Thaw Sonication:** This method allows you to rupture and reject an SUV while keeping the solute in equilibrium between the interior and exterior. This process increases the entrapment volume and efficiency. This method will lead to the formation of vesicles within and between lamellae. By up to 30%, this method raises the trapping volume.

- ➢ **French Pressure Cell:** Extrusion MLV is extruded through a French pressure cell using a small aperture. It is important to note that the proteins do not look as haughty when using the French press vesicle method as opposed to sonication. It's interesting to note that French press vesicles seem to retain entrapped solutes from sonication or detergent treatment for a longer amount of time than SUVs do. The method demands cautiously handling unstable items. The technique provides several advantages over the Sonication method. The resulting liposomes are around the size of sonicated SUVs. The method's drawbacks include the challenging high-temperature requirement and the very small working quantities (about 50 mL as the maximum). Frozen-to-thawed liposome SUVs thaw out quickly and gradually defrost. Rapid sonication with LUV disperses aggregated materials. Unilamellar vesicles are created when SUVs fuse during the freezing and thawing processes. This type of synthesis is greatly hampered by increasing the medium's ionic strength and phospholipid concentration. The encapsulation efficiencies that were discovered ranged from 20 to 30 percent.

- ➢ **Dried Reconstituted Vesicles:** Extrusion Through a tiny opening in a French pressure cell, MLV is extruded. The French press vesicle approach has the benefit that the proteins do not appear as haughty as they do during the essential phase of sonication. It is intriguing to see that French press vesicles, which are produced by sonication or detergent removal, have a propensity to maintain memories of encapsulated solutes far longer than SUVs. The technique calls for the deft

handling of delicate materials. When compared to the sonication process, the method has a number of benefits. After being sonicated, the resulting liposomes are SUV-sized. Its limitations include the method's tiny working volumes (a 50 mL maximum) and inability to maintain the high temperature.

> **Freeze-Thaw Liposome:** SUVs have rapid freezing and sluggish defrosting. Quick sonication with LUV quickly disperses aggregated materials. Unilamellar vesicles are created as a result of the SUV fusing during the freezing and thawing processes. This type of synthesis is significantly hampered by increasing the medium's ionic strength and phospholipid concentration. The obtained encapsulation efficacies were between 20 and 30 percent.

2. Mechanical Dispersion:

- **Ultrasonic Method:** SUVs between 15 and 25 um in diameter are manufactured using this method. An aqueous phospholipid dispersion can be ultrasonically processed using either a probe solicitor or a bath solicitor. Unlike probe solicitors, which are used for small volumes requiring high energy, bath solicitors are used for large volumes.

3. Solvent Dispersion:

- **Ethanol Injection:** It's a simple process. In this method, a surplus of saline or a similar aqueous medium is swiftly infused with an ethanol solution of the lipids. The ethanol is diluted with water, and the phospholipid molecules are evenly dispersed throughout the medium. A large number of SUVs are produced by this technique.

- **Ether Injection:** Injection of ethanol is similar to this technique. At the vaporization temperature of an organic solvent, it involves gently injecting an immiscible organic solution into an aqueous phase. There is virtually little possibility of oxidative damage because the lipids are handled delicately during this procedure. It takes a long time, and careful monitoring is required when adding the lipid solution.

- **Reverse Phase Evaporation:** To manufacture liposomes with a high aqueous space-to-lipid ratio and the capacity to entrap a sizable amount of the provided aqueous material for the first time, this technique advanced liposome technology. The basis for reverse-phase evaporation is the creation of inverted micelles. Sonication is used to combine an organic phase, which solubilizes the amphiphilic molecules, and a buffered aqueous phase, which includes the water-soluble molecules that will be enclosed in the liposomes, to produce these inverted micelles. The organic solvent gradually departs the solution, causing these inverted micelles to become viscous and gel. At a key point in this process, the gel state collapses, causing some of the inverted micelles to be disrupted.

  To create the water-in-oil emulsion, a two-phase system containing phospholipids in an organic solvent, such as isopropyl ether, diethyl ether, or a combination of isopropyl ether and chloroform, is first quickly sonicated. The organic solvents separate at low pressure, resulting in the production of a thick gel. The liposomes take on their ultimate shape as leftover solvent is discharged via continuing

rotational evaporation under lowering pressure. In a medium with low ionic strength, such as 0.01 M NACl, this method can generate high encapsulation efficiencies of up to 65%. Micro, macro, and gigantic substances have all been encapsulated using it. The method's main drawback is the exposure of the materials to organic solvents and brief sonication.

4. **Detergent Solubilisation:** In this procedure, detergents that interact with phospholipid molecules are used to bring the phospholipids into close contact with the aqueous phase. A micelle is a name for the resulting structure. Many millions of different molecules make them up. The critical micelle concentration, or CMC, is the point at which micelles begin to form in water that contains an adequate amount of detergent. The detergent molecule is present in a free solution below CMC. Micelle is formed in large quantities when the detergent molecule is dissolved in water with a concentration greater than CMC. More detergent is integrated into the bilayer as the concentration of detergent applied rises, up to a point where the detergent's lamellar structure changes into a spherical form.

- **Size Transformation**

  Freeze-Thaw Extrusion Method: The freeze-thaw extrusion method extends the conventional DRV procedure. The film process produces liposomes, which are vortexed with the solute to be captured until the entire film is suspended. The resulting MLV is then vortexed once more and frozen in warm water. After two cycles of freezing and thawing and overtaxing, the sample is extruded three times. eight further extrusions and six freeze-thaw cycles come next. The solute equilibrates between the interior and exterior of the SUV throughout this process, and the liposomes themselves fuse and grow to produce a huge unilamellar vesicle by extrusion method (LUVET). Using this method to encapsulate proteins is routine practice.

- **The Rehydration – Dehydration Method:** During this procedure, the SUV is enclosed in the dry buffer that has been previously rehydrated with the aqueous solution containing the chemical to be trapped. Consequently, a highly separated dispersion of solid lipids is produced. The recommended method is frequently freeze drying. The vesicles are then hydrated after that. By using this method, oligo lamellar vesicles, which are typical of liposomes, are produced.

VIII. CHARACTERIZATION

It is necessary to characterize the liposome after production and before using it in an immunoassay. The three main types of examination are physical, chemical, and biological; the physical method comprises several factors including size, shape, surface area, release profiles, etc. Chemical characterization comprises the research that determines the potency and purity of different liposomal ingredients. A formulation's appropriateness and safety for in vivo use for therapeutic purposes can be determined through biological characterization.

1. **Biological Characterization:**

Table 4 : Biological Characterization

Characterization parameters	Instrument for analysis
Sterility	Aerobic/anaerobic culture
Pyrogenicity	Rabbit fever response
Animal toxicity	Monitoring survival rats

2. **Chemical Characterization:**

Table 5: Chemical Characterization

Characterization parameter	Instrument analysis
Phospholipid concentration	HPLC / barrel assay
Cholesterol concentration	HPLC / cholesterol oxide assay
Drug concentration	Assay method
Phospholipid peroxidation	UV absorbance
Phospholipid hydrolysis	HPLC / TLC
Cholesterol auto-oxidation	HPLC / TLC
Antioxidant degradation	HPLC / TLC
PH	PH meter
Osmolality	Ohmmeter

3. **Physical Characterization:**

Table 6: Physical Characterization

Characterization parameter	Instrument for analysis
Vesicle shape Surface morphology	TEM and SEM
Vesicle size and size distribution	Dynamic light scattering, TEM
Surface charge	Free flow electrophoresis
Electrical surface potential and surface PH	Zeta potential measurement and PH-sensitive probes
Lamellarity	$P^{31}NMR$
Phase behaviour	DSC, freeze-fracture electron microscopy
Percent capture	Mini column centrifugation, gel exclusion
Drug release	Diffuse cell/dialysis

IX. STABILIZATION OF LIPOSOMES

Liposome stability ought to be comparable to that of traditional pharmaceutical formulations. The ability of the delivery system in the prescribed formulation to stay within the specified or predetermined limits for the specified period is the definition of stability for any pharmaceutical product. Chemical stability requires guarding against the oxidation of

unsaturated sites in the lipid chain as well as the hydrolysis of ester bonds in phospholipid bilayers. Chemical instability results in physical instability or drug leakage from the bilayers, fusion, and eventually vesicle aggregation.

Effective formulation and lyophilisation are two strategies that can be used to improve liposomal stability. Formulation entails choosing the right lipid composition, concentrating bilayers, and adding chemicals to the aqueous phase including cry protectants, antioxidants, and buffers. Cholesterol and sphingomyelin can be added to the formulation to reduce drug permeability and leakage, whereas phosphatidic glycerol and other charge-containing lipids can be introduced into liposome bilayers to reduce fusion. Buffers with a neutral PH can reduce hydrolysis while adding anti-oxidants like sodium ascorbate can reduce oxidation Nitrogen purging solution reduces the amount of oxygen potential when processing.

The following precaution is often necessary for the production of stable liposomal medicinal products:
1. Processing with new, clean lipids and solvents
2. Keeping away from high temperatures and a lot of shear forces
3. Preservation of low oxygen potential
4. Using cheaters or antioxidants for metals
5. Formulating at a neutral PH
6. The application of cry protectant when freeze drying

X. PURIFICATION OF LIPOSOMES

Liposomes are often purified using centrifugation, gel filtration chromatography, and dialysis. Sephardi -50 is the most often used material in chromatographic separation. You could utilize a hollow fibre dialysis cartridge when using the dialysis procedure. SUV in normal saline can be separated by centrifuging at 200000 g for 10 to 20 hours using the centrifugation method. MLV is separated by centrifuging for less than an hour at a speed of 100,000 gm.

XI. EVALUATION OF LIPOSOMES:

To guarantee its predictable in vitro and in vivo performance, liposomal formulation and processing are described for a given function. The three main categories of the characterization parameter for evaluation purposes are physical, chemical, and biological parameters.

Size, shape, surface area, lamellar, phase behaviour, and drug release are only a few of the parameters evaluated by physical characteristics.The tests that determine the potency and purity of different lipophilic ingredients are considered to be part of chemical characterization.

Biochemical characterization involves formulation safety and appropriateness for therapeutic use.Following are some parameters:

1. **Vesicle Shape And Lamellarity:** Electron microscopy techniques can be used to evaluate vesicle shape. Using freeze-fracture electron microscopy and p-31 nuclear

magnetic resonance analysis, lamellarity of vesicles is assessed, such as the number of bilayers present in liposomes.

2. **Vesicle Size And Size Distribution:** The literature describes a variety of methods for figuring out size and size distribution. These include field flow fractionation, gel permeation, and gel exclusion, as well as optical, fluorescence, and electron microscopy and laser light scattering photon correlation spectroscopy. Since electron microscopy enables one to observe each liposome and receive precise information on the profile of the liposome population over the full range of sizes, it is the most accurate approach for determining the size of a liposome. Sadly, it takes a lot of time and calls for equipment that may not always be available. In comparison, the laser light scattering approach is relatively quick and easy to use, but it has the drawback of evaluating an average liposome quality. These approaches all need pricey tools. Gel exclusion chromatography procedures are advised if only a rough concept of the size range is needed. This is because the only costs involved are those of the buffers and gel material. Liposome form, size, and stability have been examined using atomic force microscopy, a relatively recent development in microscopic methods. Most approaches employed in size, shape, and distribution studies can be divided into four categories: microscopic, diffraction, scattering, and hydrodynamic techniques.

XII. MICROSCOPIC TECHNIQUES

1. **Optical Microscopy:** The microscopic approach helps determine the size of the big vesicle and uses bright field, phase contrast, and fluorescence microscopes.

2. **Negative Stain TEM:** Negative stain TEM and scanning electron microscopy are the two main electron microscopic methods used to evaluate liposome form and size. The latter approach is less favoured. Bright regions against a dark backdrop are depicted by negative stain electron microscopy. Ammonium moly date, phosphotungstic acid (PTA), and uranyl acetate are the negative stains employed in TEM analysis. Uranyl acetate is cationic, PTA is anionic, and ammonium moly date is anionic.

3. **Cry – transmission electron microscopy techniques (cry – TEM):** This method has been used to clarify the size and surface appearance of vesicles.

XIII. DIFFRACTION AND SCATTERING TECHNIQUES

1. **Laser Light Scattering:** Brownian motion of particles in solution or suspension causes intensity fluctuations in scattered laser light, which can be analysed over time using a technique called photon correlation spectroscopy (PCS). Small particles disperse more quickly than large particles, hence the pace at which the intensity of scattered light varies as well. Thus, it is possible to measure the translational diffusion coefficient (D), which can then be used to determine the mean hydrodynamic radius (Rh) of particles. The approach can be used to analyse particles as small as 3 nm.

2. **Hydrodynamic Techniques:** This method makes use of an ultracentrifuge and gel permeation. To differentiate between SUVs and radial MLVs, exclusion chromatography on large pure gels was developed. Large vesicles, however, typically have a diameter of 1-3 um and are maintained on the surface of the column rather than entering the gel. As a

quick and practical method for estimating roughly the size distribution of liposome synthesis, a thin layer chromatography system using agarose beads has been developed. A physical blocking of the agarose gel's pores, as is the case with more traditional column chromatography, was not mentioned as being a potential issue with this method.

XIV. ENCAPSULATION EFFICIENCY AND TRAPPED VOLUME

These control the quantity and pace of water-soluble substances being trapped in the aqueous compartment of liposomes.

1. **Encapsulation Efficiency :** It describes the percentage of the aqueous phase and hence the percentage of pharmaceuticals that are finally captured during the creation of liposomes, and is often given as a percentage of entrapment/mg lipid. The mini-column centrifugation method and the protein aggregation method are two approaches used to evaluate encapsulation efficiency. Liposomes are typically purified and separated on a small scale using minicolumn centrifugation. When using the mini-column centrifugation technique, the hydrated gel is poured into a barrel of a 1 ml syringe without a plunger that has a Whitman GF/B filter pad connected. A centrifugation tube is used to support this barrel. To remove the extra saline solution from the gel, this tube is spun for three minutes at 2000 rpm. After centrifugation, the gel column ought to be dry and free of the barrel's side. Then the collected saline is taken out of the collection tube. To expel the void volume containing the liposomes into a centrifugation tube, the column is spun at 2000 rpm for three minutes after being applied dropwise to the top of the gel pad. The elute is then taken out and placed away for testing.

2. **Trapped Volume:** It's a significant factor that controls how vesicles look. The amount of water that is trapped within several lipids is known as the trapped or internal volume. the range for this is 0.5 to 30 microliters/micromole. To calculate trapped/internal volume, a variety of materials are used, such as fluorescent, radioactive markers, and spectroscopically inert fluid.

The best method for determining internal volume is to measure the amount of water directly. To do this, swap out the external medium for a spectroscopically inert fluid, and then use NMR to measure the water signal.

By scattering lipid in an aqueous solution containing a no permeable radioactive solute, trapped volume can also be calculated experimentally. The remaining activity per lipid is then evaluated after removing external radioactivity by centrifugation to determine the percentage of solute trapped.

XV. PHASE RESPONSE AND TRANSITIONAL BEHAVIOUR

It is being researched how different phase transitions in lipid bilayers and liposomes affect the triggered release of drugs or the stimulus-mediated fusion of liposomal components with the target cell. Understanding phase transitions and the fluidity of phospholipid membranes is crucial for both producing and using liposomes since these membranes' phase behaviour affects a variety of features, including permeability, fusion, aggregation, and protein binding.

Freeze-fracture electron microscopy has been used to assess the phase change. Differential scanning colorimeter study provides a more thorough verification (DSC).

XVI. DRUG RELEASE

With the aid of precisely calibrated in vitro diffusion cells, the process of drug release from liposomes may be evaluated. Using in vitro assays to foretell the pharmacokinetics and bioavailability of the medicine before using pricey and time-consuming in vivo investigations can help the liposome-based formulation. An assay that determined intracellular drug release caused by liposome degradation in presence of mouse-liver lysosome lysate was used to determine the bioavailability of the drug, and another assay that determined dilution-induced drug release in buffer and plasma was used as a predictor for the pharmacokinetic performance of liposomal formulations.

XVII. TARGETING OF LIPOSOMES

1. **Passive Targeting:** Typically delivered liposomes are quickly removed from the bloodstream and absorbed by reticuloendothelial cells in the liver and spleen, as their name implies. Therefore, when liposomes are sent to the macrophages, the ability of the macrophages can be utilized. This was proven by the efficient transport of liposomal antimicrobials to macrophages.

   As an initial stage in the development of immunity, liposomes are being employed to target antigens to macrophages. For instance, in rats, i.v. injection of liposomal antigen induced a spleen phagocyte-mediated antibody response, while liposome-associated antigen did not.

2. **Active Targeting:** The positioning of the targeting agents on the liposomal surface must be such that the interaction with the target, or the receptor, can be tallied, like a plug and socket. Physical preparation of the liposome ensures that the connector's lipophilic portion anchors into the membrane as it forms. the liposome's hydrophilic component, to which the targeting chemical should be attached in a sterically proper manner so that it may bind to the receptor on the cell surface. Bringing up the use of active targeting is possible.

3. **Immune Liposomes:** These are conventional or covert liposomes that have antibodies or another type of recognition sequence attached. The liposome is guided to a particular antigenic receptor found on a particular cell by the antibody that has bonded to it. Cell-cell recognition and adhesion are mediated by a glycoprotein or glycolipid cell surface component.

4. **Magnetic Liposomes:** The magnetic iron oxide. In their delivery sites, these liposomes can be guided by an external vibrating magnetic field.

5. **Temperature or Heat-Sensitive Liposomes:** Created in a way that their transition temperature is only a bit higher than body temperature. After arriving, the area was externally heated to cause the medication release.

XVIII. APPLICATION OF LIPOSOMES

1. **Enhancing the Effectiveness of Entrapment:** Medication loading into nanoparticles, particularly liposomes, is known to boost the therapeutic ratio of the entrapped drug by enabling selective transport of appropriate quantities of the drug to the site of action while limiting its delivery to no target (normal) tissues. However, in various experimental/clinical scenarios, the therapeutic impact of many liposomal formulations is diminished, at least in part, due to poor drug entrapment. The development of active "remote" drug loading techniques, which enable the encapsulation of several weak bases or weak acid medicines with extremely high drug-to-lipid ratios, has increased the commercial effect of liposomes (encapsulation efficiency up to 90 percent. The pH or chemical makeup of the liposomes' interior aqueous compartment is changed in the active loading approach to facilitate effective retention. Medicines contained in the liposomes. This technique was frequently used for the effective trapping of medications including doxorubicin, daunorubicin, and vincristine. Although the aforementioned approach has shown to be effective in some situations, active loading is not thought to be a universal method because many medications, such as paclitaxel or ciprofloxacin, cannot be remotely loaded into liposomes due to their high hydrophobicity or lack of an ionisable group, cannot be done so.

2. **Control Drug Release Rate:** It is common knowledge that the medicine that is captured inside liposomes is not accessible until it is released. To maximize the therapeutic efficacy of liposomal systems, the release rate of the liposomal payload must be optimized. Generally speaking, liposomes should allow for the administration of a sufficient concentration of bioavailable medication within the target tissue, at a suitable rate, for a sufficient amount of time, while holding the drug during transit to the target region (i.e., no premature release). have stated that despite the effective intratumor delivery of cisplatin in a mouse tumour model, the liposomes did not exhibit any therapeutic promise. They explained these inconsistent outcomes by the liposomes' inability to discharge a minimum cytotoxic concentration at the tumour tissue of cisplatin. In an orthotropic malignant mesothelioma tumour model, we observed comparable outcomes following intrapleural delivery of cholesterol-containing liposomes loaded with pemetrexed. It has been shown that the encapsulated medication's physicochemical characteristics and the liposomal membrane composition both affect how the drug is released from the liposomes. Drug release from liposomes was demonstrated to be decreased by adding cholesterol to the liposomal membrane, which has the effect of stiffening the membrane, and by changing the phospholipid bilayer from a fluid phase to a solid phase. Drugs with exceptionally low octane/buffer partition coefficients showed prolonged liposomal retention, whereas compounds with partition coefficients between 0.3 and 1.7 were quickly released. It's interesting to note that the particular drug entrapped influences the effect of the drug-to-lipid ratio on drug release. A higher drug-to-lipid ratio in the instance of doxorubicin led to a shorter half-life of drug retention. 46) In contrast, vincristine and irinotecan were held for a longer period in liposomes with larger drug-to-lipid ratios; a 10-fold increase in release half-life was noted when drug-to-lipid ratio increased from 0.05 to 0.6 (w/w).

3. **Liposome in Ocular Therapy:** The treatment of disorders of both the anterior and posterior segments has seen extensive use of liposomes. The list of eye diseases includes proliferative Vitroo retinopathy, keratitis, corneal transplant rejection, uveitis,

Andendophthalmitis. The primary cause of blindness in developed nations is retinal disease. A monoclonal antibody-directed vehicle and a genetic transfection vector are both made of liposomes.

Table 7: Drugs in Ocular Therapy

Drug	Vehicle	Result
Tomfool maleate	Chitosan coated liposome	Reduced i.e.,
acetazolamide	nanoparticles	Better permeability
brinzolamide	nanoparticles	Increase eye penetration rate
Dorzolamide hydrochloride	Nano liposome	Reduced i.e.
Tombolo maleate	Liposome	Reduced i.e.

4. **Liposome in Tumour Therapy:** Anticancer medication long-term therapy causes several severe adverse effects. With few adverse effects, liposomal therapy has revolutionized the field of cancer treatment. It targets tumour cells specifically. Because they can circulate for a longer period and can extravagate in tissue with increased vascular permeability, it has been claimed that small, stable liposomes are passively targeted to various tumours.

Table 8: Liposomes in Tumour Therapy

Drug	Targeted site	preparation	Marketed product
Doxorubicin	Kaposi sarcoma	Liposome	Lapdog
Doxorubicin	Refractory tumour	Liposome	Most
Doxorubicin	Metastatic breast cancer	Liposome	Doxia
Mitoxantrone	Treatment of solid tumours and lymphoma	Liposome	LEM – ETU
Doxorubicin	Metastatic breast cancer	Liposome	MM 302
Docetaxel	Anti-cancer agent	Liposome	Doxorubicin
Analysis	Kaposi sarcoma Refractory breast cancer	Liposome	Liposome kanamycin
Cisplatin	Anti-cancer agent	liposome	lipoplatin

5. **Liposome as Anti-Infective Agents:** The liver and spleen are home to intracellular pathogens such as protozoal, bacterial, andfungal organisms; as a result, medicinal agents can be sent to these organs using liposomes as vehicle systems.

Table 9: Liposomes as Anti-Infective Agents

Drug	Formulation	Targeted Organism
Penicillin G	liposomes	Staphylococcus aureus
Gentamicin	Liposomes	Pseudomonas aeruginosa
Triclosan	Liposomes	Streptococcus orals
Vancomycin	Liposomes	Staphylococcus aureus
Amikacin	Liposomes	Pseudomonas aeruginosa
Revamping	Cationic Nano liposomes	Staphylococcus epidermidis
Amphotericin B	Liposomes	Candida albinos
Clarithromycin	Liposomes	Pseudomonas aeruginosa
Gentamicin	Liposome combined beta TCP scaffold	Staphylococcus aureus
metronidazole	Mannosylated liposome	Staphylococcus aureus

6. Available Liposomel Formulation in Market:

Table 10: Available Liposomel Formulation in the Market

Drug	Application	Commercial Name	Composition of Liposome
Amikacin	Bacterial infections	Miasma	HSPC/CH/DSPG
Adriamycin (doxorubicin)	Stomach cancer	-	DPPC/CH
Ampicillin	Listeria monocytogenes	-	CH/PC/PS 5:4:1 CH: DSPC: DPPG 10:10:1
Kanamycin	Kaposi's sarcoma, Breast cancer, Leukaemia	Kanamycin	liposomes
Amphotericin B	Systemic fungal infection	AmBisomes	HSPC/CH/DSPG
All-trans-retinoic acid	Acute Proyelocytic leukemia,lymphoma	Atragens	Liposomes
Muramyl dipeptide	immunostimulator	-	DSPC/PS 1:1
Ciprofloxacin	Pseudomonas aeruginosa	-	DPPS
Clodronate	Macrophage suppression	-	PC/CH
Cyclosporin	immunosuppressor	-	PC/CH
Doxorubicin	Breast cancer	EVACET	-
Lipid A	immunoadjuvant	-	liposomes

REFERENCE

[1] Torchilin VP. Recent advances with liposomes as pharmaceutical carriers. Nat. Rev. Drug Discov., 4, 145–160 (2005).

[2] Goyal P, Goyal K, Vijaya Kumar SG, Singh A, Katare OP, Mishra DN. Liposomal drug delivery systems—clinical applications. Acta Pharm., 55, 1–25 (2005).

[3] Al-Jamal WT, Kostarelos K. Liposomes: from a clinically established drug delivery system to a nanoparticle platform for ttheranosticnanomedicine. AA Chem. Res., 44, 1094–1104 (2011).

[4] Plotnick AN. Lipid-based formulations of amphotericin B. J. Am. Vet. Med. Assoc., 216, 838–841 (2000).

[5] Blume G, Cevc G. Molecular mechanism of the lipid vesicle longevity in vivo. Biochim. Biophys. Acta, 1146, 157–168 (1993).

[6] Caliceti P, Veronese FM. Pharmacokinetic and biodistribution properties of poly(ethylene glycol)–protein conjugates. Adv. Drug Deliv. Rev., 55, 1261–1277 (2003).

[7] Han Y., Gao Z., Chen L., Kang L., Huang W., Jin M., Wang Q., Bae Y.H. Multifunctional oral delivery systems for enhanced bioavailability of therapeutic peptides/proteins. *Acta Pharm. Sin. B.* 2019;9:902–922. DOI: 10.1016/j.apsb.2019.01.004. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[8] Mirtaleb M.S., Shahraky M.K., Ekrami E., Mirtaleb A. Advances in biological nano-phospholipid vesicles for transdermal delivery: A review on applications. *J. Drug Delivery Sci. Technol.* 2021;61:102331. doi: 10.1016/j.jddst.2021.102331. [CrossRef] [Google Scholar]

[9] Mehta P.P., Ghoshal D., Pawar A.P., Kadam S.S., Dhapte-Pawar V.S. Recent advances in inhalable liposomes for treatment of pulmonary diseases: Concept to clinical stance. *J. Drug Delivery Sci. Technol.* 2020;56:101509. doi: 10.1016/j.jddst.2020.101509. [CrossRef] [Google Scholar]

[10] Yusuf H., Ali A.A., Orr N., Tunney M.M., Mc Carthy H.O., Kett V.L. Novel freeze-dried DDA and TPGS liposomes are suitable for nasal delivery of the vaccine. *Int. J. Pharm.* 2017;533:179–186. doi: 10.1016/j.ijpharm.2017.09.011. [PubMed] [CrossRef] [Google Scholar]

[11] Liu W., Hou Y., Jin Y., Wang Y., Xu X., Han J. Research progress on liposomes: Application in food, digestion behavior, and absorption mechanism. *Trends Food Sci. Technol.* 2020;104:177–189. doi: 10.1016/j.tifs.2020.08.012. [CrossRef] [Google Scholar]

[12] Himeno T., Konno Y., Naito N. Liposomes for Cosmetics. In: Sakamoto K., Lochhead R.Y., Maibach H.I., Yamashita Y., editors. *Cosmetic Science and Technology.* Elsevier; Amsterdam, The Netherlands: 2017. pp. 539–549. [Google Scholar]

[13] Niu M., Lu Y., Hovgaard L., Guan P., Tan Y., Lian R., Qi J., Wu W. Hypoglycemic activity and oral bioavailability of insulin-loaded liposomes containing bile salts in rats: The effect of cholate type, particle size and administered dose. *Eur. J. Pharm. Biopharm.* 2012;81:265–272. DOI: 10.1016/j.ejpb.2012.02.009. [PubMed] [CrossRef] [Google Scholar]

[14] Li C., Zhang Y., Wan Y., Wang J., Lin J., Li Z., Huang P. STING-activating drug delivery systems: Design strategies and biomedical applications. *Chin. Chem. Lett.* 2021;32:1615–1625. doi: 10.1016/j.cclet.2021.01.001. [CrossRef] [Google Scholar]

[15] Forssen E.A. The design and development of DaunoXome® for solid tumor targeting in vivo. *Adv. Drug Delivery Rev.* 1997;24:133–150. doi: 10.1016/S0169-409X(96)00453-X. [CrossRef] [Google Scholar]

[16] Kalyane D., Raval N., Maheshwari R., Tambe V., Kalia K., Tekade R.K. Employment of enhanced permeability and retention effect (EPR): Nanoparticle-based precision tools for targeting of the therapeutic and diagnostic agent in cancer. *Mater. Sci. Eng. C Mater. Biol Appl.* 2019;98:1252–1276. doi: 10.1016/j.msec.2019.01.066. [PubMed] [CrossRef] [Google Scholar]

[17] 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 Pharm. Sin. B.* 2021;11:2265–2285. DOI: 10.1016/j.apsb.2021.03.033. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[18] Hashemi M., Shamshiri A., Saeedi M., Tayebi L., Yazdian-Robati R. Aptamer-conjugated PLGA nanoparticles for delivery and imaging of cancer therapeutic drugs. *Arch. Biochem. Biophys.* 2020;691:108485. DOI: 10.1016/j.abb.2020.108485. [PubMed] [CrossRef] [Google Scholar]

[19] Fernandes M.A., Eloy J.O., Luiz M.T., Junior S.L.R., Borges J.C., de la Fuente L.R., Luis C.O.S., Marchetti J.M., Santos-Martinez M.J., Chorilli M. Transferrin-functionalized liposomes for docetaxel delivery to prostate cancer cells. *Colloids Surf. A.* 2021;611:125806. DOI: 10.1016/j.colsurfa.2020.125806. [CrossRef] [Google Scholar]

[20] Kang T., Gao X., Hu Q., Jiang D., Feng X., Zhang X., Song Q., Yao L., Huang M., Jiang X., et al. iNGR-modified PEG-PLGA nanoparticles that recognize tumor vasculature and penetrate gliomas. *Biomaterials.* 2014;35:4319–4332. DOI: 10.1016/j.biomaterials.2014.01.082. [PubMed] [CrossRef] [Google Scholar]

[21] Liang H., Zou F., Liu Q., Wang B., Fu L., Liang X., Liu J., Liu Q. Nanocrystal-loaded liposome for targeted delivery of poorly water-soluble antitumor drugs with high drug loading and stability towards efficient cancer therapy. *Int. J. Pharm.* 2021;599:120418. doi: 10.1016/j.ijpharm.2021.120418. [PubMed] [CrossRef] [Google Scholar]

[22] Chen Q., Gao M., Li Z., Xiao Y., Bai X., Boakye-Yiadom K.O., Xu X., Zhang X.-Q. Biodegradable nanoparticles decorated with different carbohydrates for efficient macrophage-targeted gene therapy. *J. Control. Release.* 2020;323:179–190. DOI: 10.1016/j.jconrel.2020.03.044. [PubMed] [CrossRef] [Google Scholar]

[23] Pattni B.S., Chupin V.V., Torchilin V.P. New Developments in Liposomal Drug Delivery. *Chem. Rev.* 2015;115:10938–10966. doi: 10.1021/acs.chemrev.5b00046. [PubMed] [CrossRef] [Google Scholar]

[24] . Fan Y., Mariola M., Zhang K. Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. *J. Pharm. Biomed. Anal.* 2021;192:113642. doi: 10.1016/j.jpba.2020.113642. [PubMed] [CrossRef] [Google Scholar]

[25] Wang N., Chen M., Wang T. Liposomes used as a vaccine adjuvant-delivery system: From basics to clinical immunization. *J. Control. Release.* 2019;303:130–150. DOI: 10.1016/j.jconrel.2019.04.025. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[26] Barenholz Y. Doxil®—The first FDA-approved nano-drug: Lessons learned. *J. Control. Release.* 2012;160:117–134. DOI: 10.1016/j.jconrel.2012.03.020. [PubMed] [CrossRef] [Google Scholar]

[27] Dicko A., Kwak S., Frazier A.A., Mayer L.D., Liboiron B.D. Biophysical characterization of a liposomal formulation of cytarabine and daunorubicin. *Int. J. Pharm.* 2010;391:248–259. doi: 10.1016/j.ijpharm.2010.02.014. [PubMed] [CrossRef] [Google Scholar]

[28] Ye Q., Asherman J., Stevenson M., Brownson E., Katre N.V. DepoFoam™ technology: A vehicle for controlled delivery of protein and peptide drugs. *J. Control. Release.* 2000;64:155–166. DOI: 10.1016/S0168-3659(99)00146-7. [PubMed] [CrossRef] [Google Scholar]

[29] Large D.E., Abdelmessih R.G., Fink E., Auguste D.T. Liposome composition in drug delivery design, synthesis, characterization, and clinical application. *Adv. Drug Delivery Rev.* 2021;176:113851. doi: 10.1016/j.addr.2021.113851. [PubMed] [CrossRef] [Google Scholar]

[30] Guimarães D., Cavaco-Paulo A., Nogueira E. Design of liposomes as drug delivery system for therapeutic applications. *Int. J. Pharm.* 2021;601:120571. doi: 10.1016/j.ijpharm.2021.120571. [PubMed] [CrossRef] [Google Scholar]

[31] He Y., Qin L., Huang Y., Ma C. Advances of Nano-Structured Extended-Release Local Anesthetics. *Nanoscale Res. Lett.* 2020;15:13. doi: 10.1186/s11671-019-3241-2. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[32] Alving C.R., Beck Z., Matyas G.R., Rao M. Liposomal adjuvants for human vaccines. *Expert Opin. Drug Deliv.* 2016;13:807–816. doi: 10.1517/17425247.2016.1151871. [PubMed] [CrossRef] [Google Scholar]

[33] Li Z., Perkins W., Cipolla D. Robustness of aerosol delivery of amikacin liposome inhalation suspension using the eFlow® Technology. *Eur. J. Pharm. Biopharm.* 2021;166:10–18. DOI: 10.1016/j.ejpb.2021.05.021. [PubMed] [CrossRef] [Google Scholar]

[34] Myocet. [(accessed on 1 June 2021)]. Available online: https://www.ema.europa.eu/en/medicines/human/EPAR/myocet-liposomal-previously-myocet

[35] Signorell R.D., Luciani P., Brambilla D., Leroux J.C. Pharmacokinetics of lipid-drug conjugates loaded into liposomes. *Eur. J. Pharm. Biopharm.* 2018;128:188–199. DOI: 10.1016/j.ejpb.2018.04.003. [PubMed] [CrossRef] [Google Scholar]

[36] Nogueira E., Gomes A.C., Preto A., Cavaco-Paulo A. Design of liposomal formulations for cell targeting. *Colloids Surf. B.* 2015;136:514–526. DOI: 10.1016/j.colsurfb.2015.09.034. [PubMed] [CrossRef] [Google Scholar]

[37] Kohli A.G., Kierstead P.H., Venditto V.J., Walsh C.L., Szoka F.C. Designer lipids for drug delivery: From heads to tails. *J. Control. Release.* 2014;190:274–287. DOI: 10.1016/j.jconrel.2014.04.047. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

[38] Liu Y., Mei Z., Mei L., Tang J., Yuan W., Srinivasan S., Ackermann R., Schwendeman A.S. Analytical method development and comparability study for AmBisome® and generic Amphotericin B liposomal products. *Eur. J. Pharm. Biopharm.* 2020;157:241–249. DOI: 10.1016/j.ejpb.2020.09.008. [PubMed] [CrossRef] [Google Scholar]

[39] Vyxeos Liposomal (Previously Known as Vyxeos) [(accessed on 20 June 2021)]. Available online: https://www.ema.europa.eu/en/medicines/human/EPAR/vyxeos-liposomal

[40] Takechi-Haraya Y., Matsuoka M., Imai H., Izutsu K., Sakai-Kato K. Detection of material-derived differences in the stiffness of egg yolk phosphatidylcholine-containing liposomes using atomic force

microscopy. *Chem. Phys. Lipids.* 2020;233:104992. DOI: 10.1016/j.chemphyslip.2020.104992. [PubMed] [CrossRef] [Google Scholar]u

[41] Liposomal drug delivery, a novel approach: PLARosomes Arkadiusz Kozubek, Jerzy Gubernator, Ewa Przeworska and Maria Stasiuk Department of Lipids and Liposomes, Institute of Biochemistry and Molecular Biology,   University of Wrocław, St. Przybyszewskiego 63/77, 51-148 Wrocław, Poland Received: 31 July, 2000; accepted: 10 August, 2000

[42] Kamel S. Ahmed, Saied A. Hussein, Abdelmoneim H. Ali, Sameha A. Korma, Qiu Lipeng & Chen Jinghua (2018)