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Review On A Comprehensive Study Of Developing And Evaluating Liposomes.

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ABSTRACT: The many facets of liposomal formulation, such as its properties, mechanism of synthesis, and utilisation for a range of therapeutic objectives, are the main emphasis of this study. Because of its biocompatible and biodegradable properties, which make it a non-toxic carrier and a good choice for drug carriers in the treatment of disease, liposomes are the drug carrier that researchers are most interested in studying. Its bilayer vesicles of phospholipids facilitate the encapsulation of both hydrophilic and hydrophobic medications. Many advancements have been made in the creation of a liposomal formulation for the application of drug carriers in disease treatment, primarily in the areas of immunomodulation, topical therapy, gene transfer, and cancer therapy. The word "liposome" is a combination of the Greek words "lipos," which means fat, and "soma," which means body. Liposomes are spherically shaped vesicles made up of cholesterol and phospholipids that are being extensively studied as medication carriers to increase the delivery and bioavailability of medicinal medicines. Many liposome-based drug delivery systems are presently undergoing clinical trials as a result of creative advancements in liposome technology, and some of them have recently received approval for clinical usage. Their hydrophobic and lipophilic properties, along with their size, make them promising drug delivery methods.

KEYWORDS: liposomes , phospholipid-bilayer, SLV, ULV, Applications. Methods of liposomes, particle size

INTRODUTON

Alec Douglas Bangham, a British haematologist, originally discovered liposomes in 1961 at the Babraham Institute in Cambridge, England. He published his work in 1964. A.D. Bangham and R.W. Horne discovered them while testing a new electron microscope at the institute using a dry phospholipid and gram-negative stain.[1]A.D. Bangham named the "Bag Like" arrangement "multilamellar smectic mesophase" or "Banghasomes" when it formed automatically. Gerald Weissman, a close colleague, proposed the word "Liposome" to describe phospholipid bilayer vesicles that were more user-friendly.[2]Liposomes are tiny, spherical artificial vesicles that can be made from cholesterol and naturally occurring, non-toxic phospholipids prior to metabolism and excretion. In addition to being biocompatible, liposomes' size and hydrophobic and hydrophilic properties make them attractive drug delivery vehicles. Liposomes are appealing drug delivery vehicles for hydrophilic and hydrophobic pharmaceuticals due to their special capacity to entrap medications in both the lipid and aqueous phases[3] A revolutionary medicine delivery method called liposomes seeks to deliver the medication straight to the site of action. Liposomes play a key role in this field. These vesicles are created by mixing lipids with water or a buffer solution, where continuous stirring helps them form a spherical structure. One of the most common ways to make liposomes is the thin-film hydration method, using phospholipids and cholesterol as primary components.[3] This approach allows for the delivery of medications in a controlled and targeted manner, providing significant advantages in the treatment of various diseases.[4]the polyphenols have a very diverse and

complex structures ⅝ each molecule must be studied individually to determine its solubility and positioning on or within the lipid membrane(6/⅞)its permeability through the bilayer (8) (and its interactions with the membranes under different conditions.

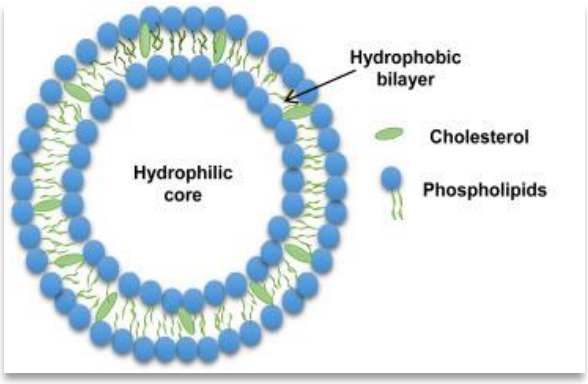


Fig.liposomes

- **Types of Liposomes :**

A) Based on Structural Features :

a)Small Unilamellar Vesicles (SUV)

b)Oligolamellar Vesicles (OLV)

c)Ultilamellar Vesicles (MLV):(9)

B) Based on Preparation Method :

1.REV: Few layered liposomes made the reverse-phase evaporation method.

2.MLV-REV: Multilayered liposomes from the same method.

3.SPLV: Stable plurilamellar vesicles.

4.FATMLV: Multilayered liposomes created by freezing and thawing.

5.VET: Vesicles prepared by extrusi

6.Vesicles formed by dehydration and rehydration.

C) Based on Composition and Application:

1.Conventional Liposomes (CL)

2.Fusogenic Liposomes (RSVE)

3.pH-sensitive Liposomes

4.Cationic Liposomes

5.Long Circulatory (Stealth) Liposomes (LCL)

6.Immuno-Liposome.[18/19]

Composition of liposomes

Liposome-related lipids and phospholipids Liposomes are spherical or multilayered spherical vesicles that form spontaneously in aqueous solutions when diacyl-chain phospholipids (lipid bilayer) self-assemble[9] An amphiphilic structure is formed by the hydrophilic head and hydrophobic tail of the bilayer phospholipid membrane [10/11]Both synthetic and natural phospholipids can be used to create liposomes[14] Particle size, stiffness, fluidity, stability, and electrical charge are all aspects of liposomes that are significantly influenced by their lipid makeup[12/13] For instance, liposomes made from naturally occurring unsaturated phosphatidylcholine, such as that found in eggs or soybeans, have limited stability and high permeability. However, liposomes based on saturated phospholipids, like dipalmitoyl phosphatidylcholine, produced stiff, nearly impermeable bilayer structures[12]

Lipids found naturally Normal cells' membrane bilayer is primarily made up of glycerophospholipids. A glycerol unit is joined to two fatty acid molecules and a phosphate group (PO42-) to form phospholipids. A tiny, necessary choline chemical molecule can also form a connection with the phosphate group[14] A variety of foods, including soy beans and egg yolks, are good providers of natural phospholipids [17]According to their polar head groups, phospholipids are categorised as

phosphatidylcholine (PC),

phosphatidylethanolamine (PE)

phosphatidylserine (PS)

phosphatidylinositol (PI),

phosphatidylglycerol (PG),

phosphatidic acid (PA).

Because of the unsaturated nature of the hydrocarbon chain, natural phospholipids are less stable than synthetic phospholipids when liposomes are being prepared.

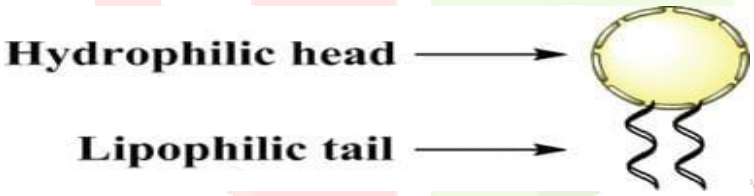
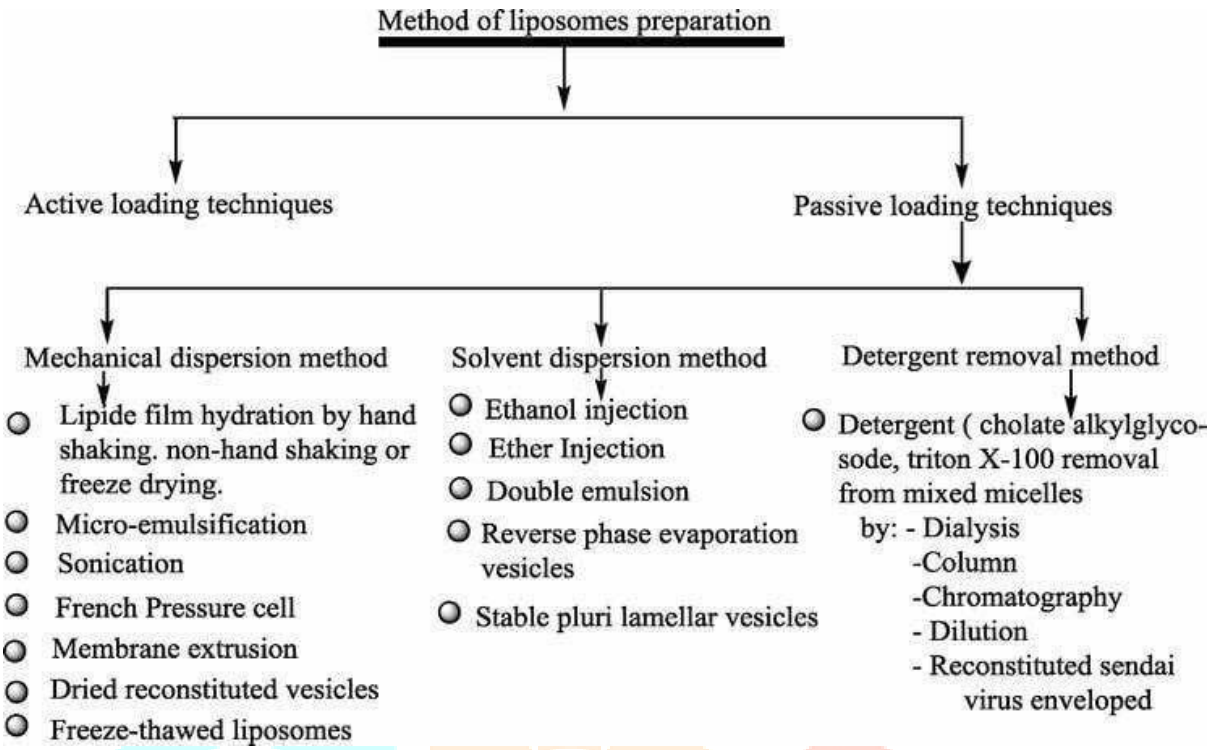


Fig. Hydrophilic &lipophilic terminals of lipid[3]

Lipids that are synthetic The polar and non-polar parts of natural phospholipids are chemically altered to create synthetic phospholipids. An infinite number of distinct and categorised phospholipids are made possible by the alteration (17]) Stearic and/or palmitic fatty acids are the main ingredients of saturated synthetic phospholipids. The many synthetic, commercial, saturated phospholipids that are typically utilised to make liposomes are depicted .

➢ **Methods preparations of liposomes:**



1 . Mechanical dispersion method :

a) Thin Film Hydration Method :

The thin film hydration method is a widely used and straightforward technique for preparing liposomes, particularly multilamellar vesicles . Preparation of the Lipid Mixture:

Phospholipids are dissolved in solvents like chloroform, dichloromethane, ethanol, or a mixture of chloroform and methanol (commonly in ratios like 3:1, 2:1, or 9:1 by

volume).Formation of a Thin Film: -This solution is placed in a rotary evaporator and dried under vacuum at a temperature between 45–60°C. This process removes the solvent, The lipid film is further dried under nitrogen gas overnight to ensure complete removal of

residual solvent. Hydration of the Lipid Film-The dried lipid film is then hydrated with an aqueous solution, typically a phosphate buffer at pH 7.2. The hydration process is carried out for 1–2 hours at 60–70°C. This method is compatible with a wide variety of lipid

mixtures, making it versatile. The lipid layers formed are not always uniform, leading to liposomes of varying sizes, which can affect their performance when analyzed later using tools like a zetasizer.[20]

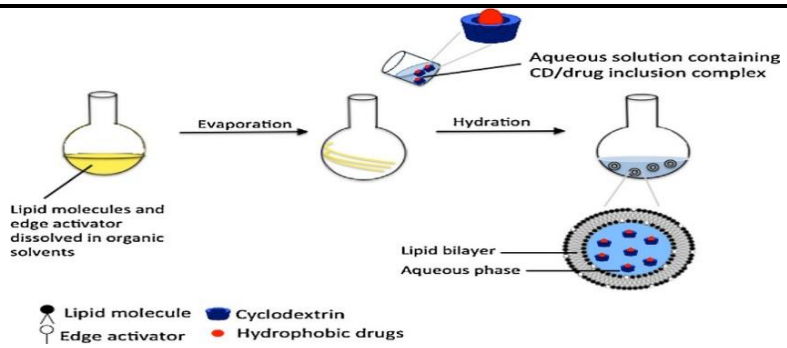


Fig.thin film hydration method

b) Sonication Method :

1.The sonication method is one of the most used.

2.Preparation of the Lipid Mixture: Natural or synthetic phospholipids are dissolved in solvents such as chloroform, dichloromethane, ethanol, or a combination of chloroform and methanol (typically in ratios like 3:1, 2:1, or 9:1 by volume).

3.Formation of a Thin Film: The solution is placed in a rotary evaporator and dried under vacuum at a temperature of 45–60°C. This removes the solvents,& a form uniform lipid film on the surface of the container. The lipid film is further dried overnight under nitrogen gas to ensure all traces of solvents are removed

4.Hydration of the Lipid Film: The dried lipid film is hydrated with an aqueous solution, usually a phosphate buffer at pH 7.2, for 1–2 hours at 60–70°C. This hydration step allows the lipid film to swell, forming larger vesicles.

5.Size Reduction Using Sonication:To create small unilamellar vesicles, the vesicle suspension is subjected to sonication. A bath sonicator is used to break down the larger vesicles into smaller, more uniform vesicles.

6.Centrifugation: After sonication, the liposome suspension is centrifuged to remove any titanium particles that may have been introduced during sonication, especially if overheating occurred.While this method is highly effective for producing SLVs. Sonication, especially if overheating occurred.While this method is highly effective for producing SLVs.

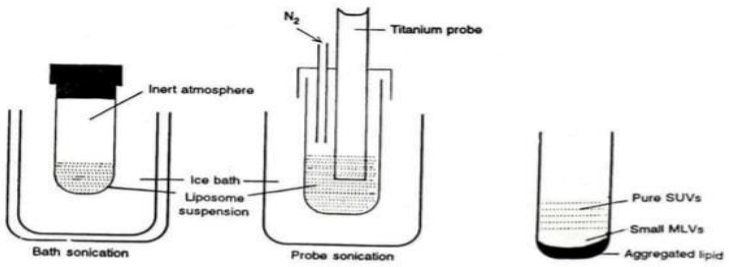


Fig.water bath & probe sonicator

C)Micro emulsification:

liposomes are prepared using a microfluidizer, a device that creates small multilamellar vesicles (MLVs) from a concentrated lipid dispersion.The process works by pumping the lipid mixture at extremely high pressure (10,000 psi) through a tiny 5-micrometer opening. This forces the fluid into micro-channels, where two streams of liquid collide at right angles at very high speeds. This intense collision efficiently breaks down and mixes the lipids, leading to the formation of small, uniform liposomes.Lipids can be introduced into the microfluidizer in two ways As large MLVs (pre-formed liposomes)As a slurry of unhydrated lipids in an organic solvent The processed fluid can be recirculated through the system multiple times until the liposomes reach the desired spherical shape and uniform size. After just one pass through the microfluidizer, the vesicle size is typically reduced to between 0.1 and 0.2 micro meters (100-200 nm).[23]

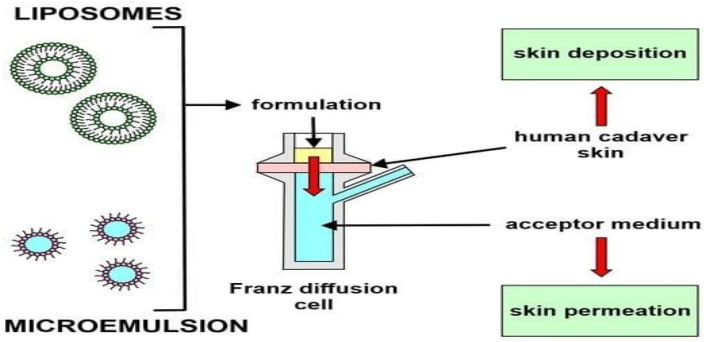


fig.microemulsion

D**) Freeze-Thaw Method:**

In the freeze-thaw method, liposomes are first prepared using the thin film hydration technique. Once formed, the liposomes are freeze-dried overnight and then thawed. This process helps control the ionic strength and phospholipid concentration in the final

liposome formulation. The repeated freezing and thawing physically disrupt the layers of the liposomal structure, resulting in a more stable and uniform ionic arrangement.

E) French Pressure Cell Method:

This method involves pushing multilamellar vesicles (MLVs) through a small orifice at high pressure (about 20,000 psi) and a temperature of 40°C. Compared to the sonication

method, this approach has several benefits: it is simple, fast, reproducible, and gentle on materials that are sensitive or unstable. While the resulting liposomes are slightly larger

than those produced by sonication, the method ensures better preservation of sensitive components and delivers consistent results.(21)

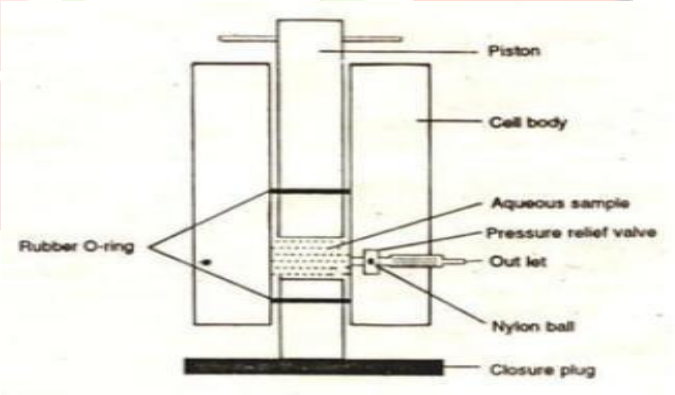


Fig.french pressure cell

2)Solvent dispersion method

In these techniques, lipids are first dissolved in an organic solvent and then brought into contact with an aqueous phase containing the materials to be encapsulated within liposomes.

a)Ethanol Injection Method : In this method, an ethanol solution of lipids is rapidly injected into an excess of saline or another aqueous medium using a fine needle. The ethanol dilutes in the water, leading to the even dispersion of phospholipid molecules throughout the medium. This technique typically produces a high yield of small unilamellar vesicles (SUVs), with an average diameter of approximately 25 nm.

b) Ether Injection Method : This method is similar to the ethanol injection technique but uses an immiscible organic solvent. The lipid solution is injected very slowly into the aqueous phase through a narrow needle, at a

temperature corresponding to the vaporization of the organic solvent. This controlled process minimizes the risk of oxidative degradation of lipids. However, the method is time-consuming and requires precise control during the injection of the lipid solution to ensure optimal resultsig.

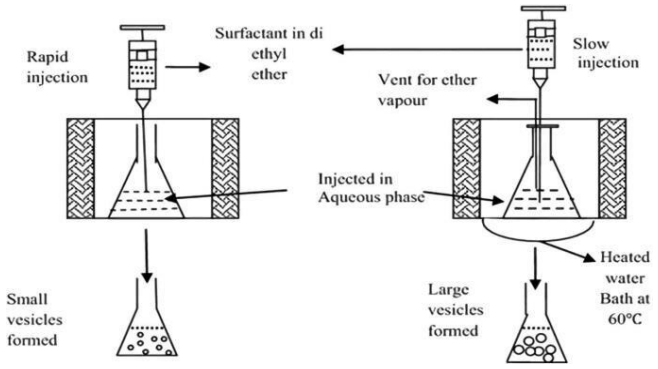


Fig.ethanol injection method & ether injection method

Method of active loading

A]Proliposome

In order to create a free-flowing granular component in a pro-liposome that, when hydrated, creates an isotonic liposomal solution, lipid and medication were coated onto a soluble carrier. 33 Large-scale, economical production of liposomes, including lipophilic medications in particular, will be encouraged by the pro-liposome strategic approach.

B]Lyophilization:

The process of lyophilization Cryodesiccation, often known as freeze-drying, is the process of removing water from materials at drastically low pressure while they are frozen. The method is widely used to dry thermolabile materials that heat-drying can destroy. This process is a crucial technique for addressing long-term stability problems with liposomal stability. Leakage of trapped materials may occur during the freeze-drying and reconstitution process. [22]

3 . Detergent Solubilization Technique:

This technique involves bringing phospholipids into close contact with the aqueous phase using detergents. Detergents interact with phospholipid molecules to form structures known as micelles. Micelles are composed of several hundred molecules. The concentration of detergent in water at which micelles begin to form is referred to as the Critical Micelle Concentration (CMC). Below the CMC, detergent molecules remain in free solution. When the detergent concentration exceeds the CMC, micelles form in significant quantities. As more detergent is added, it gets incorporated into the phospholipid bilayer. Eventually, a critical point is reached where the lamellar structure transitions into a spherical micellar form. With further increases in detergent concentration, the size of the micelles decreases.

Advantages of Using Liposomes -

1.Biodegradability: Liposomes break down naturally in the body without causing harm.

2.Controlled Release: They allow precise control over the release of their contents.

3.Mimics Natural Membranes: Their structure closely resembles that of biological membranes.

4.Enhanced Targeting: Liposomes can be designed to deliver drugs or compounds to specific cells or tissues.

5.Biocompatibility: They are well-tolerated by the body, minimizing adverse

6.Biodegradable: Liposomes break down naturally in the body, ensuring saf elimination without harmful effects[23]

Disadvantages of Liposomes:

Poor Solubility – Liposomes may not dissolve well in certain environments.

Short Lifespan – They break down quickly in the body, reducing their effectiveness.

High Production Cost – Manufacturing liposomes is expensive and complex.

Drug Leakage & Fusion – The drug inside may leak out, or liposomes may merge, affecting drug delivery.

Risk of Oxidation – The lipids used in liposomes can degrade when exposed to oxygen, reducing stability.

Potential Allergic Reactions – Some people may be sensitive to liposome ingredients.

Limited Stability – They can break down over time, making storage and handling challenging[23]

Applications of Liposomes -

1.Cancer Chemotherapy: Liposomes are used to deliver anti-cancer drugs directly to tumor sites, minimizing damage to healthy tissues.

2.Gene Therapy: Liposomes can carry genetic material, such as DNA or RNA, into cells for therapeutic purposes.

3.Vaccine Delivery: They serve as carriers for vaccines, enhancing immune responses by protecting the

active ingredients.

4.Oral Drug Delivery: Liposomes can carry drugs for oral treatment, improving absorption and bioavailability.

5.Topical Applications: Used in skincare or wound healing, liposomes can deliver active ingredients directly to the skin.

6.Pulmonary Drug Delivery: They can be used to deliver drugs directly to the lungs, improving efficacy for respiratory conditions.[24]

Marketed product of liposomes[23]

Marketed product	Drug used	Target disease	Company
AlecTM	Dry protein free poweder	Expanding lung diseases in babies	Britannia pharm, UK
VentusTM	Prosataglandin E1	Systematics infalamentry disease	The Liposomes company USA
Doxil	Doxarubucin HCl	refractory ovarian cancer	ALZA, USA
AmphotecTM	Amphotericin	Bfungal infection leishmaniasis	SEQUUS, USA
Fungizone	Amphotericin	Fungal infection	Leishmaniasis
Doxarubucin	Doxarubucin	Metastatic breast cancer	The liposomes company -USA
Novasome	Small pox vaccine	Small pox	Novavax USA

➢ **Mechanism of Liposome Formulation :**

The mechanism behind liposomal formulations is based on the unique properties of phospholipids. Phospholipids have two main parts: a hydrophilic (water-loving) "head" and a hydrophobic (water-repelling) "tail" made up of fatty acid chains. These fatty acids typically contain 10–24 carbon atoms and can have between 0 to 6 double bonds. In a liposome, the hydrophilic heads interact with water, while the hydrophobic tails tend to avoid water.

phospholipids to form layers that align in a bilayer, essentially creating a structure like a bubble. The bilayer arrangement is essential for creating the liposome, and this structure can be formed by applying energy through processes like sonication (using sound waves), homogenization (mixing), or heating[25]

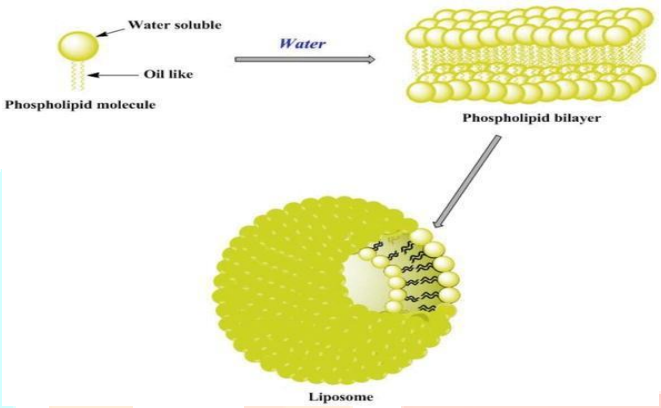


Fig. Mechanism of liposome formation

Liposomal formulations and their processing are designed for specific purposes, and they need to be carefully characterized to ensure consistent performance both in laboratory conditions (in vitro) and in living organisms (in vivo). To evaluate

liposome formulations effectively, the characterization process can be divided into three main categories: physical, chemical, and biological parameters.

1.Physical Characterization : This focuses on assessing the size, shape, surface features, and behavior of the liposomes. It also includes evaluating their lamellarity (layer structure) and how they release their contents over time.

2.Chemical Characterization : This category involves studies that help

determine the purity and potency of the lipophilic (fat-soluble) components used in the liposome formulation. Ensuring that the ingredients are of the

right quality is crucial for the effectiveness of the liposomes.

3.Biological Characterization : These parameters are used to evaluate the safety and suitability of the liposome formulation for therapeutic applications. They help in determining whether the formulation will be safe and effective for use in medical treatments.

Some of parameters Technique[26]

Characterization parameter	Analytical method
Vesicle form and surface characteristics	Electron microscopy using transmission and freeze-fracture
Vesicle size distribution(submicron and micron range) and mean size	Zetasizer, laser light scattering, dynamic light scattering, photon correlation spectroscopy, gel permeation, and gel exclusion
exclusion Charge on the surface	The electrophoresis of free-flow Surface
pH and electrical surface potential surface	Measurements of zeta potential and pH-sensitive probes
Lamellarity	31P flow -NMR, small angle X-ray scattering, Microscopy using freeze-fracture electrons Behaviour in phases electron microscopy
Behaviour in phases	electron microscopy using freeze-fracture technique, Colorimetry of differential scanning
The ratio of free drug to capture	Radiolabelling, ion-exchange chromatography, and minicolumn centrifugation Release of drugs Dialysis and diffuse cells

Conclusion:

Liposomes have emerged as a remarkable solution for this challenge. They serve as highly effective, micro-engineered vehicles for drug delivery, Offering minimal toxicity and flexibility that can be tailored to meet various needs.In India, pharmaceutical research on drug delivery systems is growing rapidly, with liposomes being a key area of focus. [27] Despite the rise of new techniques, traditional methods for liposome preparation continue to be widely used. Liposomes are now recognized as highly useful carriers for targeted drug delivery, offering versatility in how they can be used for any drug, regardless of its solubility.The potential of liposomes in drug and gene delivery is promising, and ongoing research is likely to lead to further advancements in this field In conclusion Liposomes have proven to be an effective drug delivery method for a number of illnesses, from pain management to cancer treatment. The pharmacokinetics and pharmacodynamics of the water-insoluble, poorly bioavailable, and extremely toxic medication were improved by the formation of liposomes that were biocompatible, biodegradable, and low immunogenicity.

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