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Liposomes: A Review

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ABSTRACT -
In this article , we discussed the history , basic components , types , preparation ,applications , evaluations and marketed formulations of liposomes . Liposomes are small artificial vesicles which are spherical in shape that can be made from cholesterol and natural non- toxic phospholipids . Generally , liposomes are defined as spherical vesicles with particle sizes ranging from 30 nm to several micrometers. Dr. Alec D. Bangham discovered the Liposomes at Babraham Institute in Cambridge. There are various types of liposomes which are based on Structure, Method of Preparation, Composition, Targeting concept, etc. This article provides an overview of the significant advances in synthesis of liposomes via active or passive loading. There has been an increasing number of FDA - approved liposomal -based therapeutics together with more and more undergoing clinical trials, which involve a wide range of applications in anticancer , antifungal , antibacterial, etc. Liposomes are characterized by various parameters like Vesicles size measurement , Entrapment Efficiency , Drug Content , Surface charge , Surface morphology, etc .

KEYWORDS -Liposomes ; History ; Types ; Preparation ; Characterization ; Liposome applications .

I. INTRODUCTION -

Liposomes are spherical shaped concentric vesicles derived from two Greek words lipos means ' fat ' and some means ' body ' . Liposomes are the drug carriers loaded with a great variety of molecules such as small drug molecules, proteins, nucleotides and even plasmids. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural non-toxic phospholipids. Generally, liposomes are defined as spherical vesicles with particle sizes ranging from 30 nm to several micrometers . Due to their size and hydrophobic and hydrophilic character (besides biocompatibility),Liposomes are promising systems for drug delivery.[1]

HISTORY OF LIPOSOMES -

It was the early 60's , when A.D. Bangham and his Colleagues made an observation that diffusion of univalent cations and anions out of spontaneously formed liquid crystals of lecithin is remarkably similar to the diffusion across the biological membrane . Bangham says that in Faraday's Society meetings , a number of presentations were made on electron micrographic techniques to establish apparently ubiquitous bimolecular membranes to be bi-molecular leaflets . Later, it was the beginning of ex vivo preparations of credible membrane models as first suggested by Babraham based on black lipid membrane.[2]

Thus, Davson , Horne ,Canni's Babraham , Bangham , Rodin and others were working on various aspects of artificial membrane . In 1961 , at Babraham , Bangham and his colleagues could observe these lipid mesophases following negative stains under electron microscope . The ability of these mesophases (phospholipid based liquid crystals was known latter to entrap solutes to which they were selectively permeable . These systems (mesophases) were presented as petite , putative and protean model for cell membrane and first time named as Liposomes (lipid - Sphere) by Weiss man (1965) and hence forth heralded proliferation of studies on cell membrane biophysics , structure and functions.[2]

BASIC COMPONENTS OF LIPOSOMES -

There are a number of structural and non - structural components of Liposomes . Phospholipids and Cholesterol are the major structural components amongst the various components of Liposomes . The most common phospholipid used in the formulation of Liposomes is **Phosphatidylcholine (PC)** Molecule.Phosphatidylcholine is an amphipathic molecule in which a glycerol bridge links a pair of hydrophobic acyl hydrocarbon chains , with a hydrophilic polar head group, phosphocholine. Phosphatidylcholine is also called Lecithin . It can be derived from natural and synthetic sources . Phosphatidylcholine is not soluble in water and in aqueous media.[3]

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Another major component of Liposomes is Cholesterol . Cholesterol does not by itself form a bi layer structure , but can be incorporated into phospholipid membranes in very high concentration upto 1:1 or even 2:1 molar ratios of Cholesterol to Phosphatidylcholine . Non -structural components (lipophilic/hydrophilic/amphiphilic) can be accommodated at a concentration range of 1- 10 % w/w without serious disruption of basic bi layer structure. Examples of Phospholipids are : PE (Phosphatidylethanolamine) - Cephalin, PC (Phosphatidylcholine) - Lecithin, PS (Phosphatidyl serine), PG (Phosphatidyl glycerol).[3]

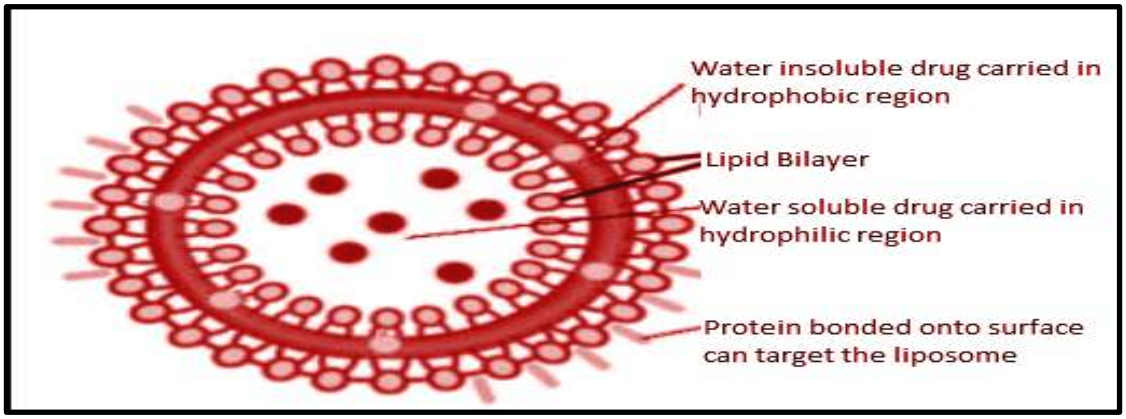
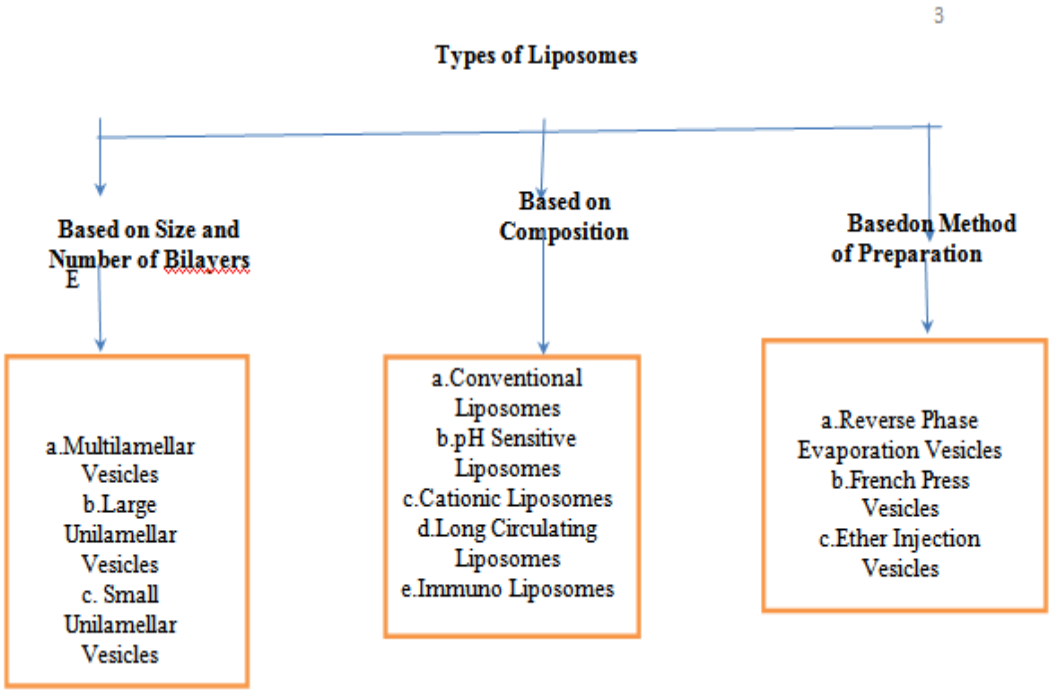


Fig. 1 : Structure of Liposome[4]

TYPES OF LIPOSOMES -: **Table No - 1**



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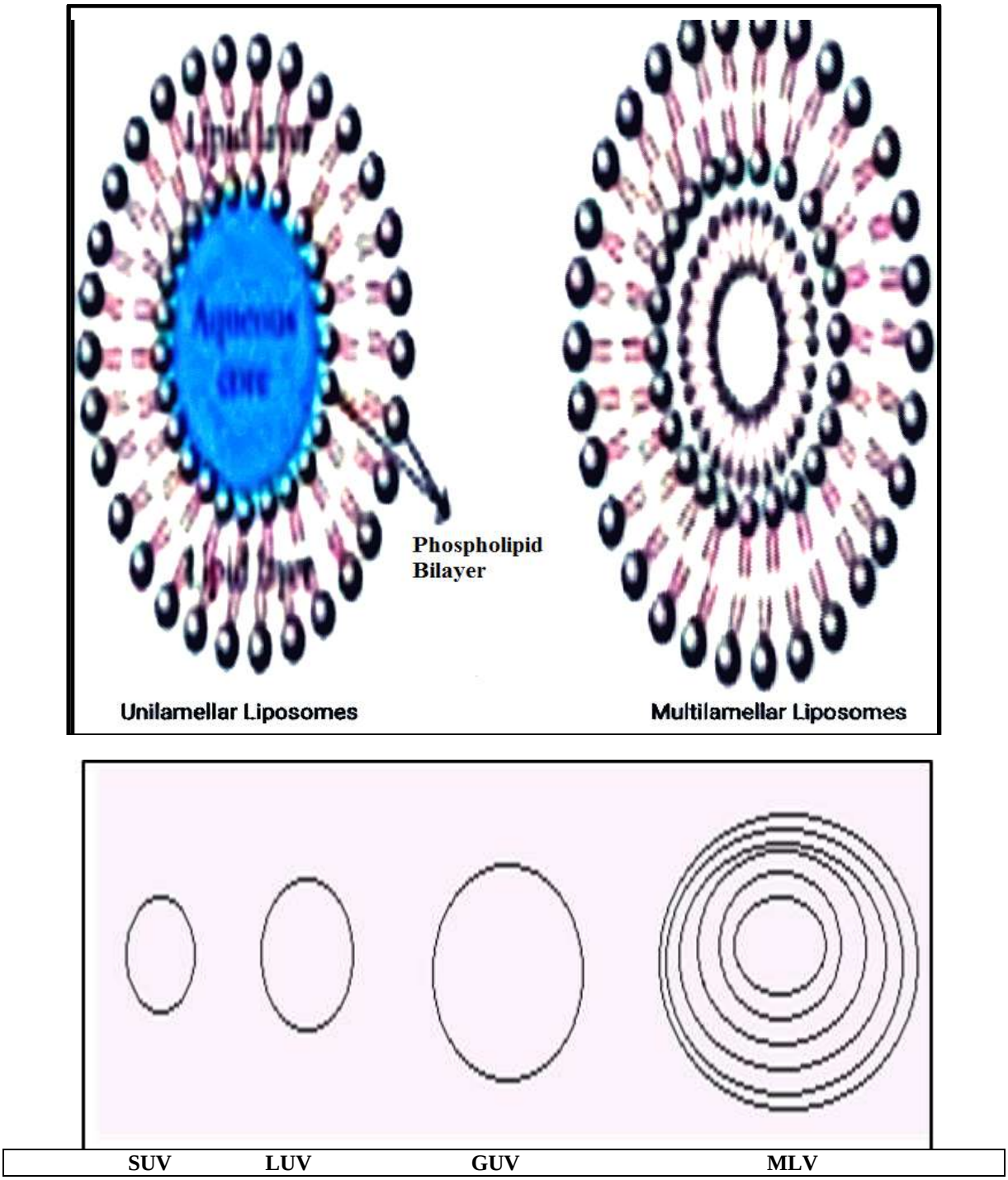

Fig. 2 : Types of Liposomes [5]

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CLASSIFICATION OF LIPOSOMES -

Table no. - 2 Classification of Liposomes [6]

Classification Based on Structural Features	Classification Based on Method of Preparation	Classification Based on Targeting Concept of Liposomes
Multilamellar Large Vesicles	Single or Oligo Lamellar Vesicles made by Reverse Phase Evaporation Method	PEGylated Liposomes
Oligo Lamellar Vesicles	Multilamellar Vesicles made by Reverse Phase Evaporation Method	Immuno Liposomes
Unilamellar Vesicles	Stable Plurilamellar Vesicles	Cationic Liposomes
Small Unilamellar Vesicles	Frozen and Thawed MLVS	Thermosensitive Liposomes
Medium Sized Unilamellar Vesicles	Vesicles prepared by Extrusion Method	
Large Unilamellar Vesicles	Vesicles prepared by French Pressure Cell	
Giant Unilamellar Vesicles	Dehydration - Re hydration Vesicles	
Multi vesicular Vesicles		

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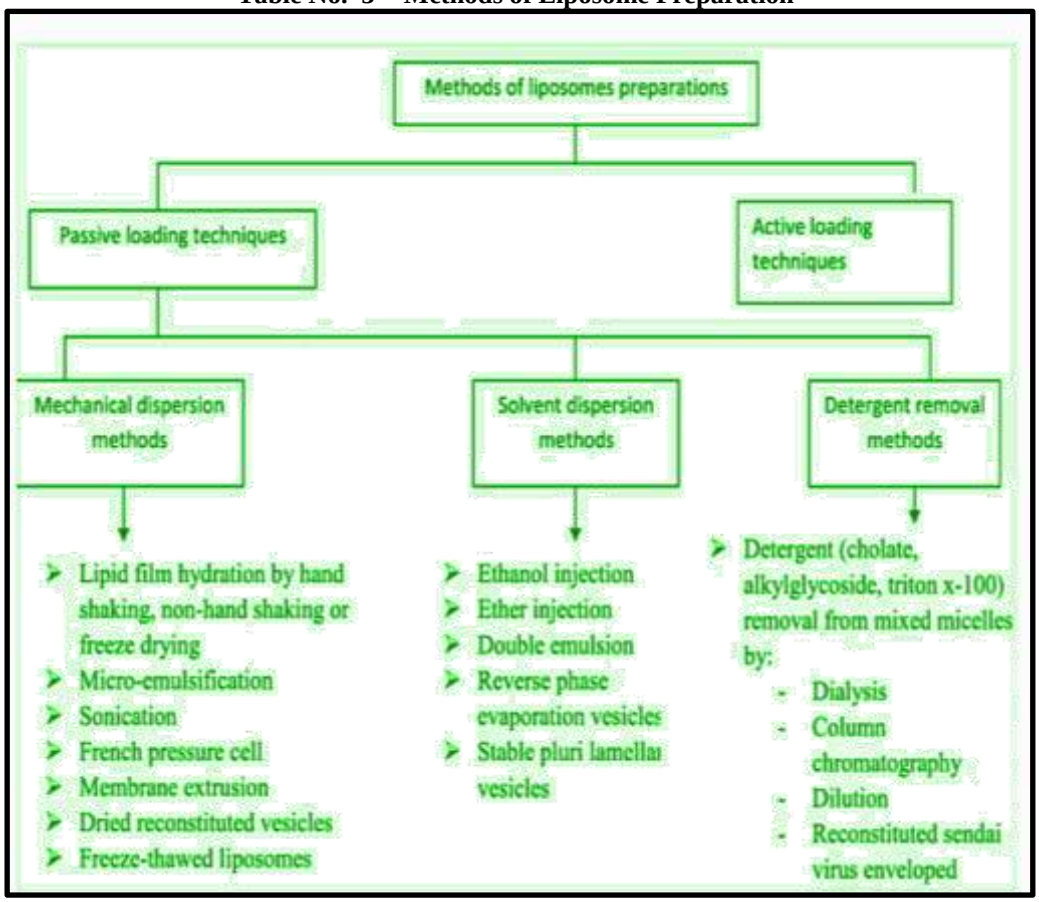
METHODS OF LIPOSOME PREPARATION - There are two loading techniques for preparation of Liposomes as follows :

- Active Loading Techniques.
- Passive Loading Techniques.

Table No. 3 - Methods of Liposome Preparation[6]



1. **Mechanical Dispersion Methods -**

A) Thin Film Hydration Method / Hand - shaking method /Bangham Method :

- This is the original method which was initially used for liposome production.
- A mixture of phospholipid and cholesterol were dispersed in organic solvent.
- Then, the organic solvent was removed by means of evaporation (using a Rotary Evaporator at reduced pressure).
- Finally, the dry lipid film deposited on the flask wall was hydrated by adding an aqueous buffer solution under agitation at temperature above the lipid transition temperature.
- This method is widespread and easy to handle,however, dispersed-phospholipid in aqueous buffer yields a population of Multilamellar Liposomes (MLVs) heterogeneous both in size and shape (1-5 µm diameter). [7]

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Lipids + Solvent (Chloroform : Methanol) in 250 ml RBF

↓

Evaporate for 15 min. above phase transition temperature

(Flush with nitrogen)

↓

Till residue dry

↓

Add 5 ml buffer containing material to be entrapped

↓

Rotate flask at room temperature at 60 rpm for 30 min. Until lipid remove from wall of RBF

↓

Milky white dispersion (stands for 2 hrs. to get Multilamellar vesicles or MLVs) .

Advantages - Simple Process.
Disadvantages -Difficulty of removing the organic solvent, the low entrapment efficiency,and the small -scale production.[8]

B) Non - hand shaking method :

- Reeves and Dowben reported a non - shaking vesicle method , where large Unilamellar vesicles can be formed with higher entrapment volume.[7]
- This is similar to the shaking method except that care is taken in swelling procedure.[7]

Lipid + Solvent

↓

Add water saturated nitrogen until opacity disappears

↓

Add bulk fluid (drug & 10 - 20 ml 0. 2 M Sucrose solution to swell)
(Flush again with nitrogen)

↓

Stand for 2 hrs. at 37 ° C do not disturb for 2 hrs.
(Swirl to yield milky dispersion)

↓

Centrifuge at 1200 rpm for 10 min. At room temperature
(MLV on surface is removed)

↓

To remaining fluid add iso - osmotic glucose solution
(Centrifuge at 1200 rpm)

↓

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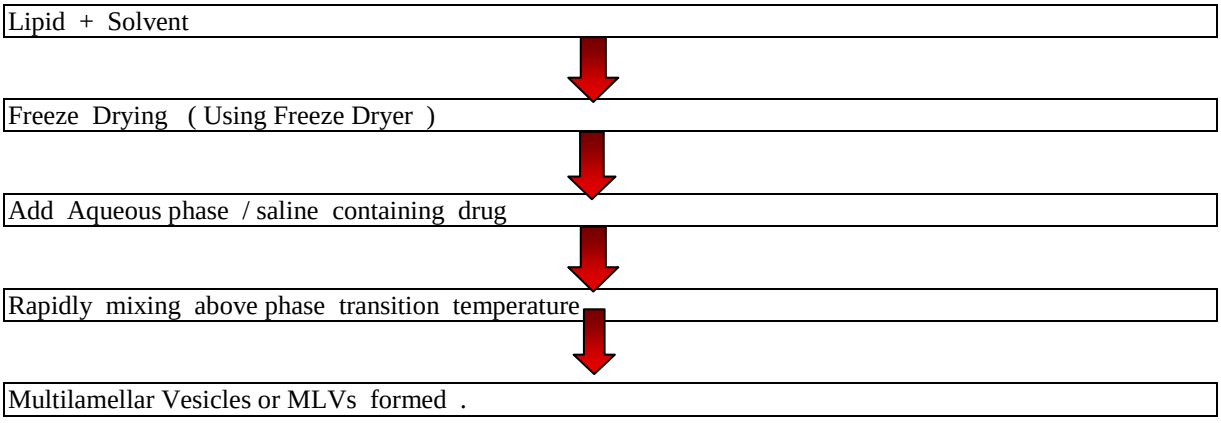
Large Unilamellar Vesicles or LUVs formed .

C) Freeze Drying :

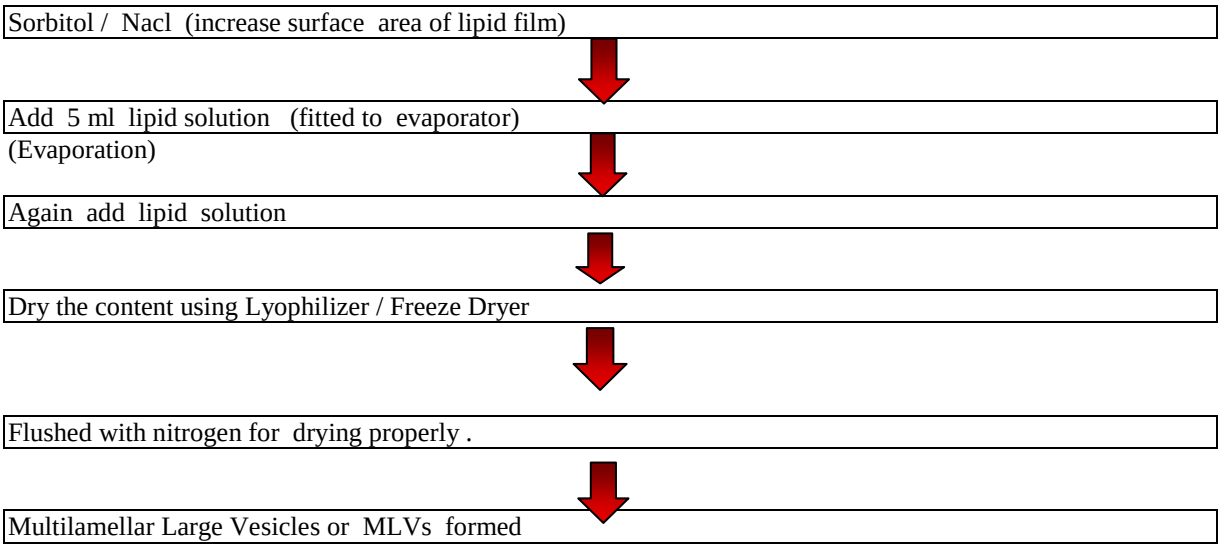
- Freeze Drying is another method of dispersing the lipid in a finely divided form where lipids dissolve in suitable solvent and freeze dried prior to addition of aqueous media.[7]
- The solvent choice depends on the freezing point which needs to be above the temperature of the condenser of the Freeze Dryer, and is inertness with regard to rubber seals which form a part of most commercial lyophilizers.[8]
- The solvent usually used is tertiary butanol.[11]
- Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at extremely low pressures.[11]
- The process is generally used to dry products that are thermolabile and would be destroyed by heat-drying.[11]

Advantage : This technique has a great potential as a method to solve long term stability problems with respect to Liposomal stability.[11]



D) Pro Liposomes :

- In this method, drug molecules and lipids both are coated with soluble carriers to form freely flowing granules in proliposomes. When hydrated results in an isotonic Liposomal suspension.[11]
- The size of the carrier influences the size and heterogeneity of the Liposomes.
- The pro-liposome approach may provide an opportunity for cost-effective large scale manufacture of Liposomes containing particularly lipophilic drugs.[11]



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- This method also overcomes the stability problems of Liposomes encountered during their storage in liquid , dry or frozen form .[11]

E) Microemulsification Liposomes (MEL) :

- Micro fluidizer is used to prepare small MLVS from concentrated lipid dispersion . Micro fluidizer pumps the fluid at very high pressure (10,000 psi, 600 -700 bar) through a 5 orifice .[2]

- Then , it is forced along defined micro channels , which direct two streams of fluid to collide together at right angles at a very high velocity , thereby effecting a very efficient transfer of energy.[2]

- The lipids can be introduced into the fluidizer , either as a dispersion of large MLVs or as a slurry of unhydrated lipids in an organic medium.[8]

- The fluid collected can be recycled through the pump and interaction chamber until vesicles of the spherical dimension are obtained.[8]

F) French Pressure Cell :

- The Heart of French Press is a Pressure cell , manufactured in stainless steel and designed to resist up to 20,000 or even 40,000 psi . Two pressure cells of different sizes are available.The large one holds up to 40 mL while the smaller one is the cell of choice and may hold < 4 mL volume.[2]

- The Pressure cell is composed of the body , containing a precision bored cylindrical cavity (the pressure chamber) with small outlet orifice (Valve) , a bottom seal , a piston , valve closure and outlet tubing .The bottom seal and piston are fitted each with the different 'O' rings , to ensure a tight seal even at very high pressures.[2]

- Liposome (or cell) dispersion is filled into the pressure chamber , the piston is inserted a short distance into the body and the cell is turned upside down . The liquid sample is introduced into the cavity.[8]

- The height of the piston is adjusted by pressing down on the outer wall of the cell , so that the chamber is completely filled with liquid. [8]

- This technique yields rather uni OligoLamellarLiposomes of intermediate size (30-80 nm in diameter depending on the applied pressure). These Liposomes are more stable as compared to sonicated Liposomes.[8]

Disadvantage : This technique is the high initial cost of purchasing an entire new system which consists of an electric hydraulic press and pressure cell .[8]

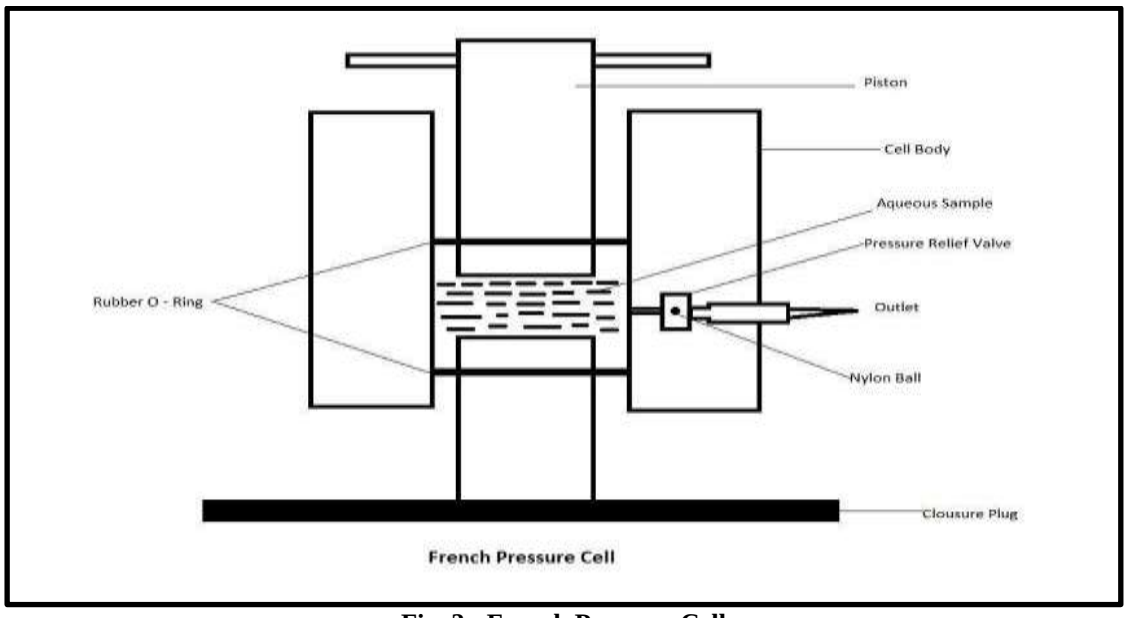

Fig. 3 : French Pressure Cell

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G) Membrane Extrusion :

- In the Membrane Extrusion method , the size of Liposomes is reduced by gently passing them through a Polycarbonate membrane filter of defined pore size .
- This can be achieved at much lower pressure (<100 psi) than required for French Pressure Cell .
- There are two types of membrane filters ,
- ❖ Tortuous path type membrane - Used for Sterile filtration .
- ❖ Nucleation track membrane - The nucleation track type is composed of a thin continuous sheet of polycarbonate.
- Used for preparation of LUVs And MLVs .

Advantage -The extrusion methods have a high reproducibility of downsizing.[10]

Disadvantages -The main disadvantage of this method is the sensitive product losses, which represent a limit for large - scale productions.[10]

H) Sonication :

- This method reduces the size of the vesicles and imparts energy to lipid suspension.[8]
- This can be achieved by exposing the MLV to ultrasonic irradiation.[8]
- There are two methods of sonication

A) using Bath sonicator : The probe sonicator is used for suspensions which require high energy in small volume. (eg: high concentration of lipids or viscous aqueous phase)

B) using Probe sonicator : The bath sonicator is used for a large volume of dilute lipids**.**

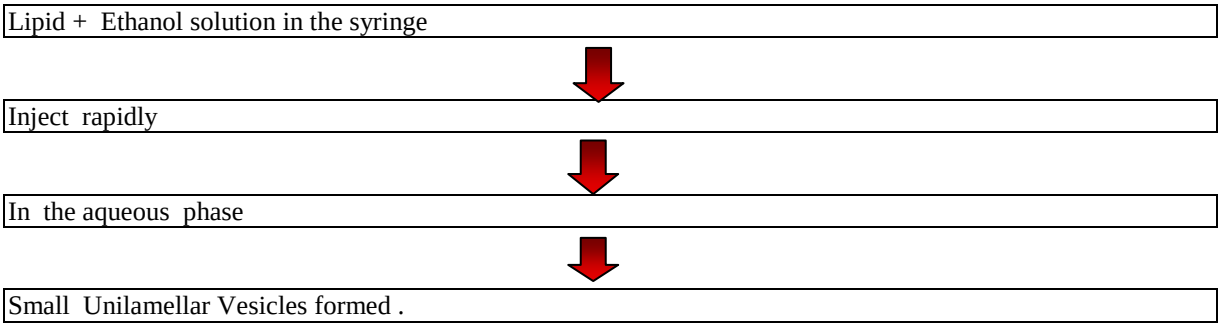
The disadvantage of Probe sonicator is contamination of preparation with metal from the tip of the probe.[10]

- By this method small Unilamellar vesicles are formed and they are purified by ultra centrifugation.[10]

2.Solvent Dispersion Methods -

a) Ethanol Injection Method :

- This method was reported by Batzri and Korn.[2]
- The Ethanol Injection method was first described in 1973.
- The main relevance of the Ethanol injection method resides in the observation that a narrow distribution of small Liposomes (under 100 nm) can be obtained by simply injecting an ethanolic lipid solution in water, in one step, without extrusion or sonication.



Advantage -This method is extremely simple and has low risk of degradation of sensitive lipids .

Disadvantage -Liposomes are very dilute , the removal of all ethanol is difficult because it forms into an azeotrope with water , probability of the various biologically active macro molecules to inactivate in the presence of even low amounts of ethanol is high.[2]

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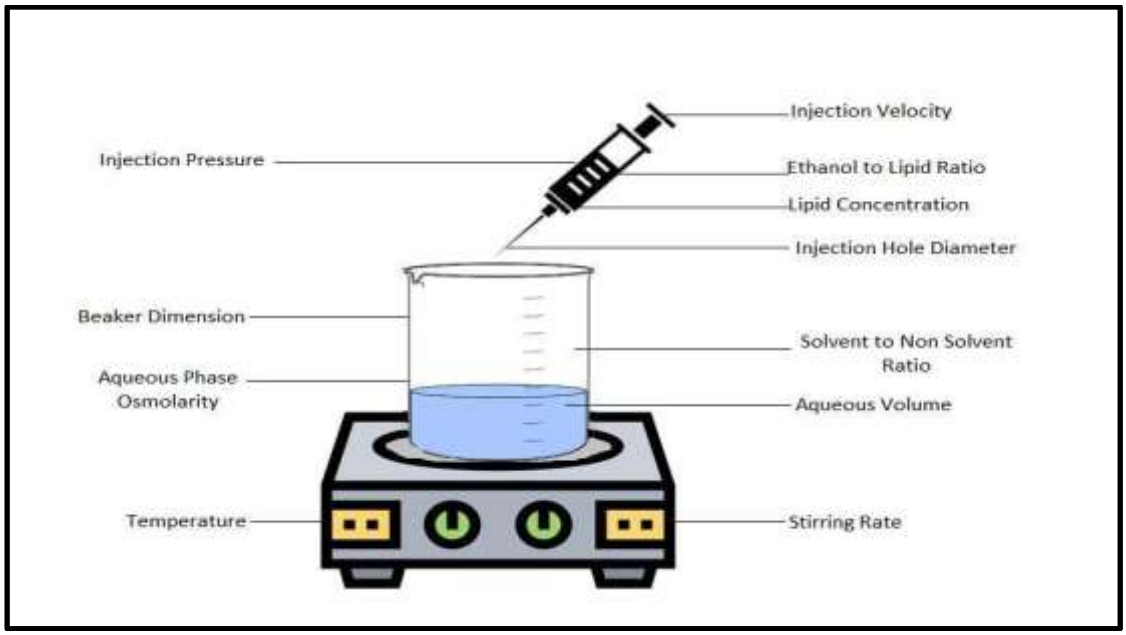

Fig. 4 : Ethanol Injection Method

b)Ether Injection Method :

- The Ether Injection method is very similar to the ethanol injection method.
- The method involves injection of ether-lipid solutions into warmed aqueous phases above the boiling point of the ether.[7]

Lipid + Ether solution in the syringe

↓

Inject slowly

↓

In the aqueous phase
(On heated water bath 60 ° C)

↓

LUVs formed .

Advantage -It has little risk of causing oxidative degradation provided ether is free from peroxides.[2]

Disadvantage - Low Entrapment efficiency.[2]

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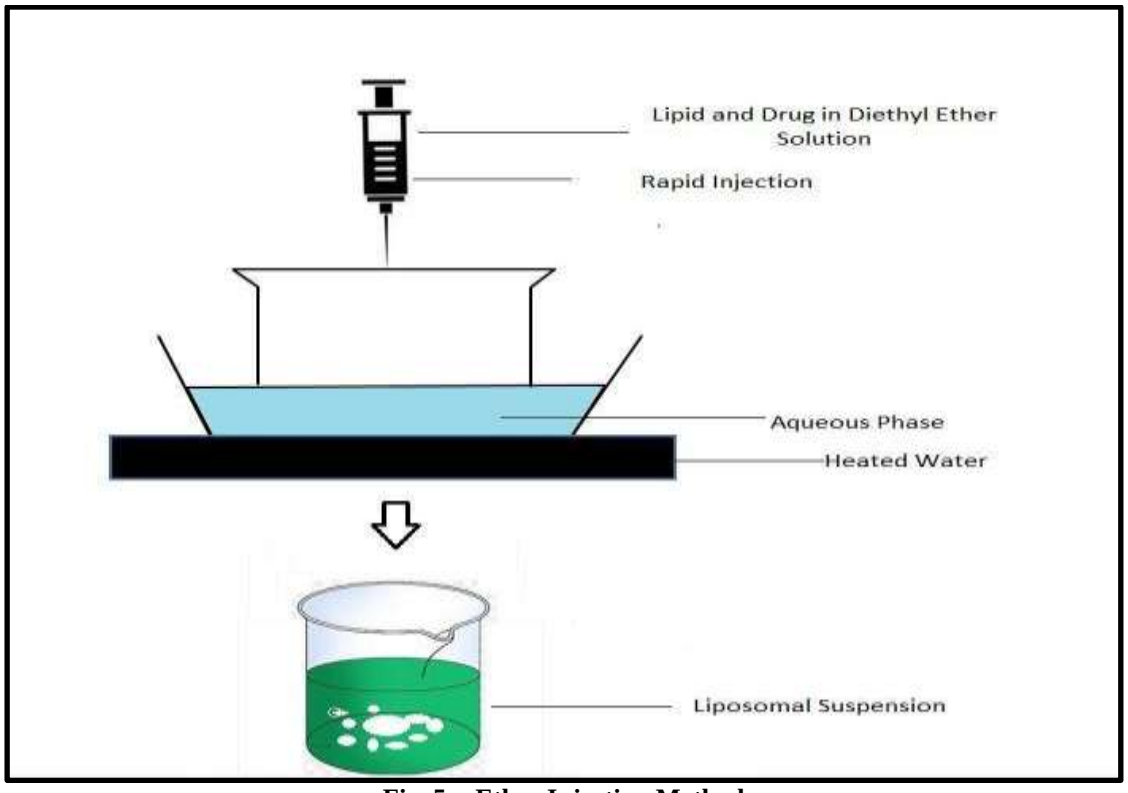

Fig. 5 : Ether Injection Method

c) Double Emulsion Vesicles :

- In this method, the outer of the liposome membrane is created at a second interface between two phases by emulsification of an organic solution in water.
- If the organic solution, which already contains water droplets,is introduced into excess aqueous medium followed by mechanical dispersion, multi-compartment vesicles are obtained.
- The ordered dispersion so obtained is described as w/o/w system (i.e. Double Emulsion).[7]

Organic Solvent + Lipid + Aq. Phase

↓

w / o emulsion

↓

Hot aqueous solution buffer

↓

Multi Compartment vesicles (w / o / w)

(Double Emulsion)

↓

LUVs

d) Reverse Phase Evaporation Vesicles :

- This method was developed by Szoka and Papahadjopoulos.[8]
- The novel key in this method is the removal of solvent from an emulsion by evaporation.[8]
- The droplets are formed by Bath sonication of mixture of the two phases, then the emulsion is

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dried - down to a semi - solid gel in a Rotary evaporator under reduced pressure.[9]

- Large Unilamellar or Oligo Lamellar vesicles are formed when an aqueous buffer is introduced into this mixture.[9]
- These vesicles have aqueous volume to lipid ratios that are 30 times higher than sonicated preparations and 4 times higher than multilamellar vesicles.[9]

Advantages - Simple Design , Suitable Encapsulation efficiency.
Disadvantages - Not suitable for encapsulation of fragile molecules due to large quantities of organic solvent use, time consuming, sterilization issue.

3.Detergent Solubilization Technique -

- Detergents associated with the phospholipid molecules and serve to screen the hydrophobic portions of molecules from water.
- The structures formed as a result of this association is known as Micelles.Upon removal of detergent , transition of mixed micelles occurs to form concentric bi-layered vesicles.
- The Detergent should have a high critical micelle concentration (CMC), so that it is easily removed.
- Liposome size and shape depend on the chemical nature of detergent, concentration and other lipids involved.
- **Methods to Remove Detergents:** Dialysis and Column Chromatography.[10]

CHARACTERIZATION OF LIPOSOMES -
The purpose of the evaluation of Liposomes is to ensure the in - vivo and in - vitro performance of Liposomes. Evaluation Parameter are categorized into three broad categories are :

i. Physical Characterization .
ii. Chemical Characterization .
iii. Biological Characterization .

✔ **Vesicle Shape -**Vesicle shape can be evaluated utilizing Electron Microscopic techniques.[10]

✔ **Lamellarity** - Lamellarity of vesicles for example number of bi layers present in Liposomes is decided to utilize freeze - fracture electron microscopy and p-31 nuclear magnetic resonance analysis.[10]

✔ **Visual Appearance -** Liposome suspension can range from translucent to milky, depending on the composition and vesicle size. If the turbidity has a bluish shade this means that vesicles in the sample are homogeneous; a flat, gray color indicates that presence of a non Liposomal dispersion and is most likely a disperse inverse hexagonal phase or dispersed microcrystallites. An optical microscope (phase contrast) can detect liposome> 0.3 µm and contamination with larger vesicles.[11]

✔ **Trapped Volume -** It is an important parameter related to Liposomes. It is aqueous entrapped volume per quantity of lipids. This can vary from 0.5 to 30 microlitres/micromol.[12]

✔ **Vesicle Size and Size Distribution** - Various techniques are used to describe the size and size distribution for e.g. Light microscopy, Fluorescent microscopy , Electron microscopy , Laser light scattering , Photon correlation spectroscopy, Field flow fractionation , Gel permeation , and Exclusion.[13]

✔ **Encapsulation Efficiency -** This determines the amount and rate of entrapment of water - soluble agents in the aqueous compartment of the liposome.[13]

✔ **Drug Release -** The component of medication discharge from liposome can be surveyed by utilizing very much aligned in -vitro dispersion cells.[13]

APPLICATIONS OF LIPOSOMES -
There are various recent applications of Liposomal drug delivery system are as follows :

A. Liposomes for Respiratory Drug Delivery System .
B. Liposomes in Nucleic Acid Therapy .
C. Liposomes in Eye Disorders .
D. Liposomes as Vaccine Adjuvant .
E. Liposomes for Brain Targeting .
F. Liposomes as Anti - Infective Agents .
G. Liposomes in Tumor Therapy.[13]

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MARKETED LIPOSOMAL FORMULATIONS -:

1. Amphotericin - B -

Table No. 4 - Liposomal Marketed Formulations[14]

Trade Name	Manufactured by
Inj. Amphotin	United Biotech
Inj. Fungisome	Life care Innovations Pvt. Ltd.
Inj. Amphocil	Criticare Laboratories Pvt. Ltd.
Inj. Ambisome	Mylan
Inj.Ampholyn	Hetero
Inj. Phosome	Cipla
Inj. Sporotar	Emcure Pharmaceuticals
Inj. Amphonex	Bharat Serums

2. **Doxorubicin -**

Table No. 5- Liposomal Marketed Formulations[15]

Trade Name	Manufactured by
Inj. Drix	Wockhardt
Inj. Nudoxa	Zydus
Inj. Lipisol	Alkem
Inj. Oncodox	Cipla
Inj. Pegadria	Intas
Inj. Lipodox	Sun Pharma
Inj. Piglit	Emcure Pharmaceuticals
Inj. Rubilong	Emcure Pharmaceuticals

II. CONCLUSION :

Almost from the time of their discovery in 1960's and the demonstration of their entrapment potential, liposome vesicles have drawn attention of researchers as potential carriers of various bio active molecules that could be used for therapeutic applications in humans and animals. After three decades of development, Liposomes are promising as

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a drug delivery vehicle with general applications. Liposomes have been recognized as therapeutic carriers in very diverse clinical fields because of their unique Physicochemical properties. Employing Liposomes as drug delivery systems provide a platform for delivering of drugs with reducing side effects and increasing their efficacy, solubility, and bio availability.[16]

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REFERENCES :

[1]. Abolfazl,A.; Rogaie, R. S.; Soodabeh, D.; Sang, W. J.; Nosratollah, Z.; Younes, H.; Mohammad, S.; Mohammad, K.; Kazem, N. K. Liposome: Classification, Preparation, and Applications. Nanoscale Res. Lett. 2013, 8, 102–110.

[2]. Vyas S.P. Dixit V.K. " Advances in Liposomal Therapeutics " CBS Publishers and Distributors Pvt. Ltd. New Delhi ; 1st edition,1,48 - 64 .

[3]. Mishra H, Chauhan V, Kumar K, Teotia D, A comprehensive review on Liposomes: a novel drug delivery system, Journal of Drug Delivery and Therapeutics. 2018; 8(6):400-404.

[4]. https://images.app.goo.gl/Z9oLGtZjeJSBPdgJA

[5]. Mane N, MohiteV, Jadhav A,Patil S,"A Review on Drug Delivery System :Liposome" International Journal of Creative Research Thoughts.2021,9(7):c662-c663.

[6]. Zaquiyya Naaz*, Dr. Kapil Kumar and Deepak Teotia. A Comprehensive Review on Liposomes. International Journal of Modern Pharmaceutical Research. 2020, 4(5), 70-74.

[7]. Laouini, A.; Jaafar-Maalej, C.; Limayem-Blouza, I.; Sfar, S.;Charcosset, C.; Fessi, H. Preparation, Characterization and Applications of Liposomes: State of the Art. J. Coll. Sci. Biotechnol. 2012, 1, 147–168. DOI: 10.1166/jcsb.2012.1020.

[8]. Jain N.K."Controlled and Novel Drug Delivery " CBS publishers and Distributors Pvt. Ltd . New Delhi , 304 - 305 .

[9]. Vishvakarma P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014; 47-55.

[10]. Kant Shashi, Kumar Satinder, Prashar Bharat, A Complete Review On: Liposomes, International Research Journal of Pharmacy, 2014; 3 (7): 10-16.

[11]. Himanshu A, Sitasharan P, Singhai AK: Liposomes as drug carriers. IJPLS 2011, 2(7):945–951.

[12]. Reddy K, Rao M, Venkatesh P, "A Review on Liposomes"International Journal of Indigenous Herbs and Drugs.2022;7(1) 8-11.

[13]. Singh A,Srivastava A ,Gupta A, "Liposomal Drug Delivery System –A Review. Journal of Applied Pharmaceutical Sciences and Research ". 2020;3 (3): 7-10.

[14]. https://www.google.com/search?q=Liposomal+Amphotericin+b+injection

[15]. https://www.google.com/search?q=Liposomal+doxorubicin+injection

[16]. Samad A ,Sultana Y, Aqil M , " Liposomal Drug Delivery Systems " An Update Review Current Drug Delivery , 2007 ; Bentham Science Publishers Ltd. 297 - 305 .