ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
Research Article
www.ijptonline.com

LIPOSOME : AN EMERGING TOOL IN DRUG CARRIER SYSTEM

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Received on 18-02-2016 *Accepted on 10-03-2016*

Abstract

Liposomes are adequate and advanced carriers and have capacity to encapsulate hydrophilic and lipophilic drugs as well as maintain them from exernal environmental condition which leads to degradation. Liposomes are colloidal lipidic bilayer vesicles which are beneath extensive exploration as drug carriers for enhancing the delivery of therapeutic agents. In consequence of new developments in liposome technology, A number of liposome based drug formulations are presently in clinical trial, and in recent times few of them have been intended for clinical use. Formulation of drugs in liposomes has rise up with new opportunity to enhance the therapeutic indices of various potent agents mostly by changing their pharmacokinetics and pharmacodynamics. This review discusses the mechanism of liposome formation , method of preparation , classification of material used, characterization , their application as well as the marketed product with its future prospectus.

Keywords: Preparation Method, Classification, Mechanism of Liposome Formulation, Stability, Drug Release, Characterization, Application of liposome.

Introduction

The liposome name is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Liposome discovered in early 1960's ny Bengham and colleagues and subsequently became the most extensively explored drug delivery system.[1,56,57] In the beginning they were used to study *in vivo* simulated biomembrane behaviour. Liposome have covered predominantly medical , even though some non-medical areas like bioreactors, catalysts, cosmetics and ecology.[2] Paul Ehrlich coined the liposome term as “magic bullet” in 20th century in which carrier system was proposed to simply carry the drug at site specific target and give its action.[3] In this review highlight on the potential applications of liposomes in drug delivery with method of preparation, classification, pharmacokinetic, biodistribution and various examples of formulations approved for clinical use, their method of preparation, targeting,

mechanism of formation, liposome component and the problems related with further development of this drug delivery system.[4] Their predominance in drug delivery and targeting has enabled them to be used as therapeutic tool in fields like tumor targeting , gene and antisense therapy ,genetic vaccination , immune-modulation ,lung therapeutics , fungal infection , and topical cosmetics products.[1]

Advantage of Liposome [5, 16]

- Liposomes used to increased efficacy and therapeutic index of drug.
- Liposome may increased stability via encapsulation.
- Liposomes posses properties like non-toxic, flexible, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations.
- Liposomes help in reducing the toxicity of the encapsulated pharmaceutical agent by avoiding the exposure of sensitive tissues to toxic drugs.
- Liposome are more flexible to couple with site-specific ligands to achieve active targeting.
- Liposome enhance and control the pharmacokinetics and pharmacodynamics parameter.
- Liposome decreased toxicity.
- Liposome enhanced activity of drugs against intracellular as well as extracellular pathogens.
- Liposomes can be made to be site specific action.
- Liposomes can entrap hydrophilic as well as hydrophobic pharmaceutical agents, in their bilayer compartment structure like outer lipid membrane and inner aqueous core.
- Liposome-incorporated pharmaceuticals are stable from the inactivating effect of external conditions,and not cause undesirable side reactions.
- Liposomes posses unique property to deliver pharmaceuticals into cells or even inside individual cellular compartments.
- The properties of liposomes Size,charge and surface can be easily changed simply by altering the lipid mixture before liposome preparation and/or by changing of preparation methods.

Disadvantage of Liposome [4 , 6 ,16]

- Liposome Production required very high.
- Liposome may cause leakage and fusion of encapsulated drug/molecules.
- Liposome posses Short half-life.

- Liposome may affect the stability of formulation.
- Due to unfavourable condition liposome content undergoes oxidation and hydrolysis like reaction.
- Liposme undergo Low solubility problem.

Structure of Liposome

Liposomes were artificially prepared vesicles made of lipid bilayer as shown in fig 1. Liposomes can be filled with both hydrophilic or hydrophobic drugs. Drug molecules can either be encapsulated in the aqueous space or intercalated into the lipid bilayer ; the exact location of a drug in the liposome will depend upon its physicochemical characteristics and the composition of the lipids .[9] Liposome were also known as concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a lipid bilayer membrane usually composed of natural or synthetic phospholipids. Liposomes used to prepare in micro particulate or colloidal carriers, mainly 0.05- 5.0 µm in diameter which form spontaneously when required amount of lipid are hydrated in aqueous media. Liposomes are consist of relatively biocompatible and biodegradable nontoxic material, and they contain an aqueous volume entrapped by one or more lipid bilayer membrane.[1]

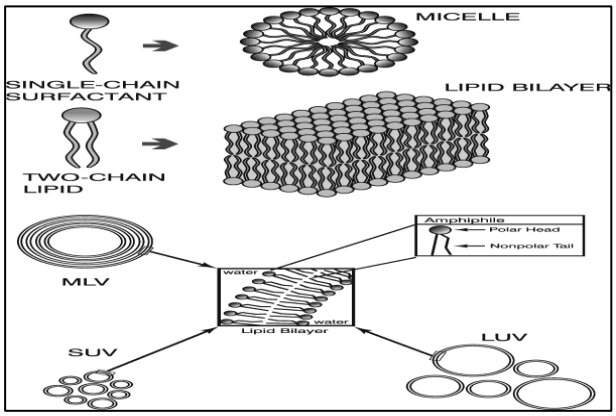


Fig No. 1: Structure of liposome.

Mechanism of Liposome Formation[2]

- Phospholipids being amphipatic (having affinity for both aqueous and polar moieties) molecules,they include a hydrophobic tail and a hydrophilic or polar head.
- The polar domain of fragment is mainly the phosphoric acid bound to a hydrophillic molecule.
- The hydrophilic and hydrophobic end/fragment within the molecular geometry of amphiphilic lipid arrange them self and organize in ordered supra-molecular structure when confronted with solvent.
- In aqueous medium like water and phosphoric acid the molecule in self assembled structure is oriented to form the structure as a polar portion of molecule remain in contact with the polar environment and simultaneously shield the non-polar part.

- In the excess of these polar lipids, liquid-crystalline phases are formed that upon dilution with an excess of water can be dispersed into relatively stable colloidal particles.
- The macroscopic structure most often formed consist of lamellar, hexagonal or cubic phases dispersed as colloidal nanoconstruct (artificial membrane) known as liposomes , hexasomes or cubosomes respectively.

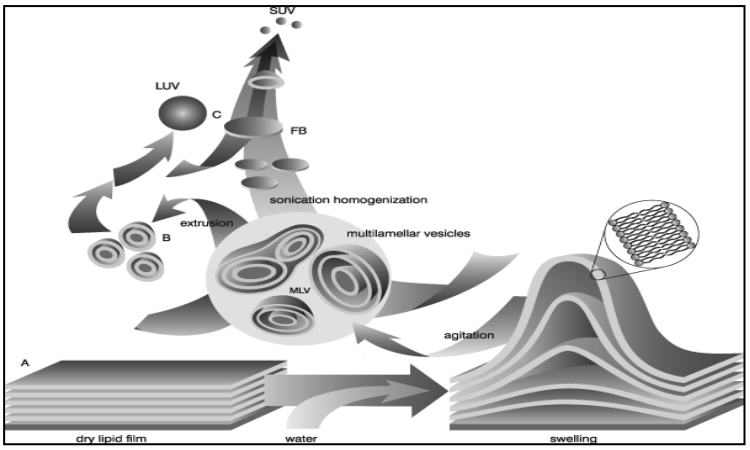


Fig No .2: Formation Of Liposome.

Classification of Liposome[8]

Table No. 1 : On structure basis, liposome classified into.

ABBREVIATION	VESICLE TYPE	MULTI VESICULAR
LUV	Large unilamellar	More than 100 nm
MUV	Medium unilamellar	More than 100 nm
SUV	Small Unilamellar	20-100 nm
GUV	Giant unilamellar	20-100 nm
OUV	Oligolamellar	0.1 to 1 micrometer
MLV	Multi lamellar	More than 0.5 micrometer
MV	Multi vesicular	more than 1 micrometer

Table No. 2 : On preparation, liposome classified into:

TYPE	SPECIFICATION
REV	Single or oligolamellar vesicle made by reverse phase evaporation method
MLV-REV	Multilamellar vesicle made by reverse phase evaporation method
SPLV	Stable plurilamellar vesicle

FATMLV	Frozen and thawed MLV
VET	Vesicle prepared by extrution technique
DRV	Dehydration rehydration method

Table NO . 3 : On the basis of composition and application, liposomes classified into:

TYPE	SPECIFICATION
Conventional liposome	Single or oligolamellar vesicle made by reverse phase evaporation method
Fusogenic liposome	Multilamellar vesicle made by reverse phase evaporation method
Cationic liposomes	Stable plurilamellar vesicle
Long circulatory liposomes	Frozen and thawed MLV
pH sensitive liposomes	Vesicle prepared by extrution technique
Immuno liposomes	Dehydration rehydration method

On the Basis of Conventional Liposome:

- Stabilize natural lecithin (PC) mixtures
- Synthetic identical, chain phospholipids
- Glycolipids containing liposome

On the basis of Speciality Liposome:

- Bipolar fatty acid
- Antibody directed liposome.
- Methyl/ Methylene x- linked liposome.
- Lipoprotein coated liposome.
- Carbohydrate coated liposome.
- Multiple encapsulated liposome

Materials Used For Preparation of Liposome

A . Phospholipids

Glycerol containing phospholipids are most frequently used element of liposome formulation and represent greater than 50% of weight of lipid in biological membranes. These are made up from phosphatidic acid. The back bone of

the molecule is glycerol moiety. At C3 position OH group is esterified to phosphoric acid. OH at C1 & C2 are esterified with long chain. Fatty acid giving rise to the lipidic nature. One of the remaining OH group of phosphoric acid may be further esterified to a broad range of organic alcohols including glycerol, choline, ethanolamine, serine and inositol. Thus the parent compound of the series is the phosphoric ester of glycerol. Phosphatidylcholine (PC) is routinely used as a neutral bulk phospholipid .

As a negatively charged lipid,phosphatidylglycerol (PC) is often selected. Finally , if it is desirable to reduce the perm eability of "fluid crystalline state" bilayers, cholesterol is added to bilayer structure.

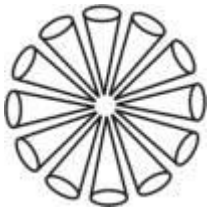
Five groups of phospholipids tthat can be used for the liposomal preparation can be discerned

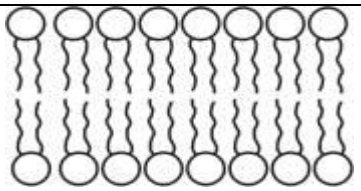
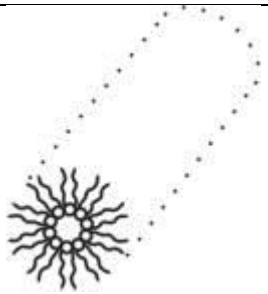
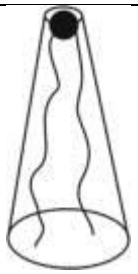
1. Phospholipids from natural soures[12]
2. Modified natural phospholipids
3. Semisyntheyic phospholipids[11]
4. Fully synthetic phospholipids
5. Phospholipids with non natural head groups

Examples of phospholipids are

- Phosphatidyl choline (Lecithin) PC
- Phosphatidyl ethanolamine (cephalin) PE
- Phosphatidyl serine (PS)
- Phosphatidyl inositol (PI)
- Phosphatidyl Glycerol (PG)

Table No. 4 : Polymorphic phases and corresponding dynamic molecular shapes of component phospholipids :

Phospholipid	Phase	Molecular shape
lysophospholipids		
	Micellar	Inverted cone
PC		
SM		

PS PG PI PA CL		
	Bilayer	Cylinder
PE (unsaturated) PA-Ca^{2+} PA (pH < 3) PS (pH < 3) CL-Ca^{2+}		
	Hexagonal	Cone

For stable liposomes, saturated fatly acids are used.Unsaturated fatty acids are not used generally.

a) Sphingolipids

Backbone is sphingosine or a related base. These are important constituents of plant and animal cells. Sphingomyelin is capable of hydrogen bonding with adjacent glyceryl lipids, thus rising order and stability of vesicle construction. [12]A head group that can vary from simple alcohols such as choline to very complex carbohydrates.

Most common

- Sphingolipids – Sphingomyelin ,Glycosphingo lipids.
- Gangliosides – found on grey matter, used as a minor component for liposome production.

This molecule consist of complex saccharides with one or more Sialic acid residues in their polar head group & therefore have one or more negative charge at neutral pH. These are included in liposomes to provide a layer of surface charged group.

b) Sterols[13]

Cholesterol & its derivatives are often included in liposomes for

- Cholesterol reduce the fluidity or microviscocity of the outer membrane.
- Cholesterol alter the packing of phospholipid molecule in the structure.
- Cholesterol enhance vesicle resistance capacity to form aggregate.

- Cholesterol decrease the permeability of the bilayer membrane to water soluble (hydrophilic) molecules
- Cholesterol also helps in the formartion of Stable membrane in the presence of biological fluids such as plasma.(This effect used in formulation of i.v. liposomes)

c) Synthetic phospholipids[14]

Saturated phospholipids

- Dipalmitoyl phosphatidyl choline (DPPC)
- Distearoyl phosphatidyl choline (DSPC)
- Dipalmitoyl phosphatidyl ethanolamine(DPPE)
- Dipalmitoyl phosphatidyl serine (DPPS)
- Dipalmitoyl phosphatidic acid (DPPA)
- Dipalmitoyl phosphatidyl glycerol (DPPG)

Unsaturated phospholipids

- Dioleoyl phosphatidyl choline (DOPC)
- Dioleoyl phosphatidyl glycerol (DOPG)

d) Polymeric materials[14]

Synthetic phospholipids including diactylenic group in the hydrocarbon chain polymerizes when exposed to U.V, it will lead to formation of polymerized liposomes which have considerably superior permeability barriers to entrapped aqueous drugs.

e.g. For other Polymerisable lipids are lipids comprising of conjugated diene, methacrylate etc.

B. Other Substances[14]

Variety of different lipids of surfactants are helps in formation of liposomes. There are various single chain surfactants which can form liposomes by mixing with cholesterol. A different type of polyglycerol and polyethoxylated mono and dialkyl amphiphiles used mainly in cosmetic preparations.Single and double chain lipids having fluoro carbon chains may form very stable liposomes.

Ionic surfactant like Steryl amine and dicetyl-phosphate incorporated into liposomes so as to impart either a negative or positive surface charge to these structures. A number of compounds having a single long chain hydrocarbon and an ionic head group which are capable of forming vesicles. These consist of quaternary ammonium salts of dialkyl phosphates.

Method of Preparation of Liposomes:[9]

Following are different methods of preparation of liposome

Liposome prepared by two techniques mainly as following

1. Active drug loading

2. Passive Drug loading

1.Passive drug loading

A. Mechanical dispersion method

- Lipid film hydration by hand shaking,non-hand shaking or freeze drying
- Micro-emulsification
- Sonication
- French pressure cell
- Membrane extrusion
- Dried reconstituted vesicles
- Freeze-thawed liposome

B.Solvent dispersion methods

- Ethanol injection
- Ether injection
- Double emulsion vesicles
- Reverse phase evaporation vesicles
- Stable plurilamellar vesicles

C.Detergent removal method

- Detergent(cholate,alkylglycoside,triton x-100)removal from mixed micelles by
- Dialysis
- Column chromatography
- Dilution
- Reconstituted sendai virus enveloped vesicles

2. Active loading

1. Passive Loading

In these passive loading technique the drug is encapsulated by incorporating an aqueous phase of a water-soluble (hydrophillic) drug or an organic phase of a lipid-soluble drug initially or at predetermined stage during the preparation of the liposomes. The huge drug encapsulation efficiency can be achieved with the help of these passive loading technique which is more suitable for lipid-soluble drugs with a high resemblance to the lipid membrane.[18] Different methods discuss under this class start with a lipid solution in organic solvent and end up with lipid dispersion in water. The a choice of component are typically combined by co-dissolving the lipids in an organic solvent and the organic solvent is then seperated by film deposition under vacuum .When residual solvent is removed, the soild lipid mixture is hydrated with the help of aqueous buffer. The lipids spontaneously swell and hydrate to form liposome. Liposomal encapsulation technology (LET) is the latest delivery method used by medical researcher to transmit drugs that act as healing promoters to the definite body organs. LET is state of art method of preparing sub-microscopic bubbles called liposome.[17]

A. Mechanical Dispersion Method

In these method variety component are mainly combined by co-dissolving the lipids in an organic solvent and after that the organic solvent is then separated by film deposition under vaccume. When all the solvent is evaporated , the solid lipid mixture is hydrated using aqueous phase . The lipids spontaneously swell and hydrate to form liposomes. Ethosomes can also be made up using these method. Ethosomes are soft, flexible lipid vesicles composed mostly of phospholipids, alcohol (ethanol or isopropyl alcohol) in relatively high concentration (20-45%) and water.[20] Clotrimazole ethosomes were formed by these method also known as ultra deformable liposome by R.G.S. Maheshwari et al.[21] Fluconazole liposomes also prepared by these method.[22]

a. Lipid Film Hydration By Hand Shaking, Non-Hand Shaking or Freeze Drying

In lipid film hyadration method , the lipids are casted as stacks of film from their organic solution by means of flash rotator evaporator in reduced pressure (or with hand shaking).These stacks of film dispersed in an aqueous phase medium like buffer solution or organic solvent. Upon addition of buffer (hydration) the lipids swell and peel off from the wall of round bottom flask(RBF) and vesiculate will form multilamellar vesicles(MLVs). The percent encapsulation efficiency as high as 30% (at 100mg lipid ml^{-1})[2]. Thermosensitive Liposomes Containing Docetaxel is prepared by this technique[19.] Pilocarpine nitrate[23] ,econazole[24], amphotericin b liposomes[25], nabumetone liposomes[26] , oxcarbazepine[27], Ketoconazole liposome[28], Tacrolimus (Fk-506)[29], calcium phosphate coated liposome[30],

Ofloxacin[31], trimethylchitosan coated insulin liposomes[32] and Multilamellar vesicles of Imiquimod[33] were prepared using these method.

Advantage

- 100% encapsulation efficiency shown by lipid soluble compounds , provided they are present in adequate quantities.
- Liposome does not alter structural composition of membrane.

Disadvantage

- Most of water-soluble compounds are washed out during swelling as only 10-15% of the total volume gets entrapped.

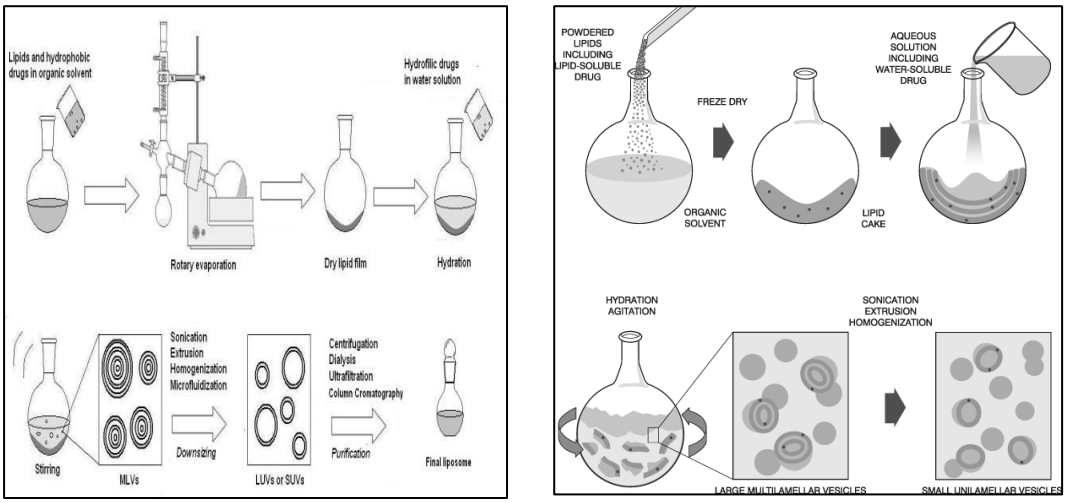


Fig No. 3: Lipid Film Hydration By Hand Shaking.

b. Micro-Emulsification

In the microfluidizer the lipids can be introduced either as a dispersion of large MLVs , or as slurry of unhydrated lipid in an organic medium like phosphate buffer or volatile solvent. The fluid collected can be recycled throughout the pump and contact chamber until vesicles of the spherical dimension are gained. Microfluidizer pupms the fluid at very high pressure (0,000psi, 600-700bar) through a 5 µm orifice. Then , it is forced along defines micro channels ,which direct two streams of fluid to collide together at right angles at a very high velocity , thereby affecting an efficient transfer of energy. The percent encapsulation efficiency is 70% (at lipid 200mg/ml concentration).[2] Small unilamellar vesicles (SUVs) composed of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipid (Avanti Polar Lipids, Alabaster, AL, USA) were prepared by the extrusion method or sonication.[34]

Advantage

- Production rate by these method is high .

- By these method high proportion of lipids (20% or more by weight) cab be process.

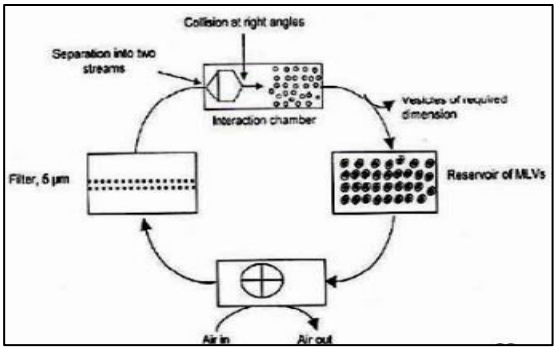


Fig No. 4 : Micro-Emulsification.

c. Sonication

There are two methods of sonication based on either probe or bath ultrasonic disintegrators.The probe is employed for small volume high concentrated lipid viscous phase while bath is employed for large volume of diluted lipids. Sonication of an MLV dispersion is accomplished by placing a test tube containing the dispersion in a bath sonicator (or placing the tip of the sonicator in the test tube in a probe sonicator) and sonicating for 5-10 min (1,00,000g) above the TC^0 of the constituent lipid.The lipid dispersion should begin to clarify to yield a slightly hazy transparent solution.This dispersion placed in ultracentrifuge tube and centrifuged at 100,00g (30 min, 20^0c) to sediment large MLVs followed ny higher speed centrifugation (1,59,000g for 3-4 h).Ciprofloxacin Hcl elastic liposome prepared by sonication method for treatment of acne.[34] R. Silva et al also study the effect of sonication in preparation of liposome[35]. Ryan Caddell and its co-worker found that liposome mean particle size controlled by sonication.[36] Machado et al also study the sonication result on preparation of liposomes containing a protein source, such as Spirulina platensis.[39]

Disadvantage

- Lipid dispersion suffer from overheating of the liposomal dispersion.
- Sonication tip in probe sonicato tend to release titanium particles into the liposomal dispersion.

Fig No. 5 : Bath Sonicator And Probe Sonicator.

d. French Pressure Cell

It consist of an electric hydraulic press and pressure cell. French pressure cell involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. An interesting comment is that French press vesicle appears to recall entrapped solutes significantly longer than SUVs do, produced by sonication or detergent removal. The method involves gentle handling of unstable materials . These French press has used to reduce the heterogeneity of populations of proteoliposomes obtained by detergent dialysis technique. Robert L. Hamilton et al prepared unilamellar liposomes using french pressure cell method and also found remarkable stability of liposomes may be due to storage of these liposomes above the transition temperature of egg PC.[41] Here . D. Lichtenberg et al shown that homogeneous preparations of quite small (315-525 A diam.) Unilamellar vesicles obtained by the use of a Power laboratory press (French press). In this apparatus, dispersions was subjected to high hydraulic pressure. It is frequently used for distintegrating chloroplast material, blood cells and other cells with rigid walls such as yeast cells.[43] Peter I. Lelkes et al prepared Small unilamellar by high pressure extrusion in a French press. Liposomes , consist of phosphatidylcholine, phosphatidylserine, and cholesterol at a molar ratio of 7:1:2, were incubated with suspensions of bovine adrenal chromaffin cells.[44]

Advantage

- Method is simple, rapid, reproducible.
- Less structural defects and instabilities in vesicles found by this technique.
- Slower and lower leakage of contents from liposome.

Disadvantage

- High initial cost of the press.
- The drawbacks of the method are that the high temperature is difficult to attain.
- The working volumes are comparatively small (about 50 mL as the maximum).

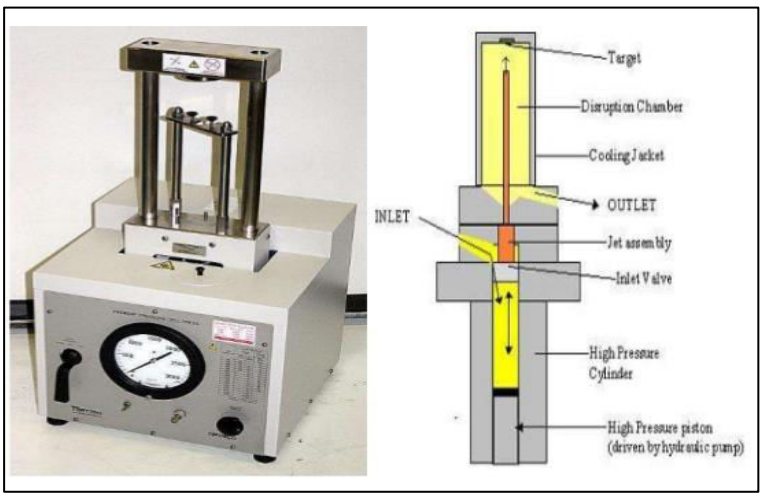


Fig No. 6 : French Pressure Cell.

e. Membrane Extrusion

In this method, phospholipids are initially introduced in a buffered saline solution to form large, multilamellar vesicles. After that vesicles are repeatedly passed through a polycarbonate membrane filter with 100 nm pores. The result vesicle have uniform sized known as unilamellar vesicles (large unilamellar vesicles, or LUV), about 100 nm in diameter. The phospholipids must be processed at a temperature above their c transition temperature (T) from gel to liquid crystalline phase. In this method the size of the vesicle can be reduced by passing through a membrane filter of defined pore size. There are two kind of membrane filter. The tortuous path type and the nucleation track type. Liposome that are larger in diameter get struck when one tries to pass them through such membrane. [15] The nucleation track type is in the form of thin continuous sheet of polycarbonate. This method can be used to form both LUVs and MLVs .It is owing to their ease of production , readily selectable vesicle diameter , batch to batch reproducibility, and freedom from solvent and/or surfactant contamination. The 30% capture volume can be obtained using high lipid concentration (300Mm pc). Leslie A. Morton et al prepared nanosized lipid vesicle by membrane extrusion.[51] Morano et a1 explained membrane extrusion with the help of suitable example.[52]

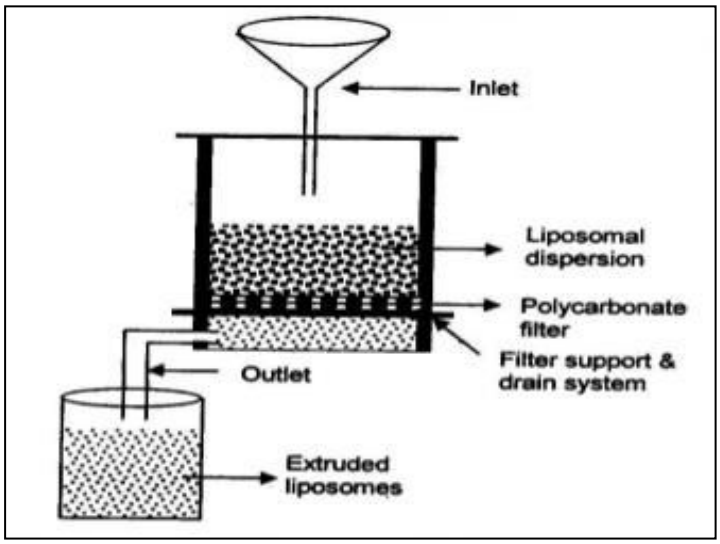


Fig No. 7 : Membrane Extrusion.

f. Dried Reconstituted Vesicles

In DRV method freeze drying of a dispersion of empty SUVs are to be done and then dispersion of it with the aqueous fluid containing the material to be entrapped. This leads to a hydration of solid lipids in finely reduced sized form. Though , the step of freeze-drying is introduced to freeze and lyophilize a performed SUVs dispersion rather than to dry the lipids from an organic solution. This leads to an ordered membrane structure as compared to random matrix structure , which on addition of water can rehydrate , fuse and reseal to form vesicles with a high encapsulation efficiency. The water soluble hydrophillic materials to be entrapped are added to the dispersion which

are empty SUVs and they are dried together , so the material for inclusion is present in the dried precursor lipid before the final step of addition of aqueous medium.

Advantage

- High entrapment of water soluble component.
- The use of mild conditions for the preparation and loading of bioactives .

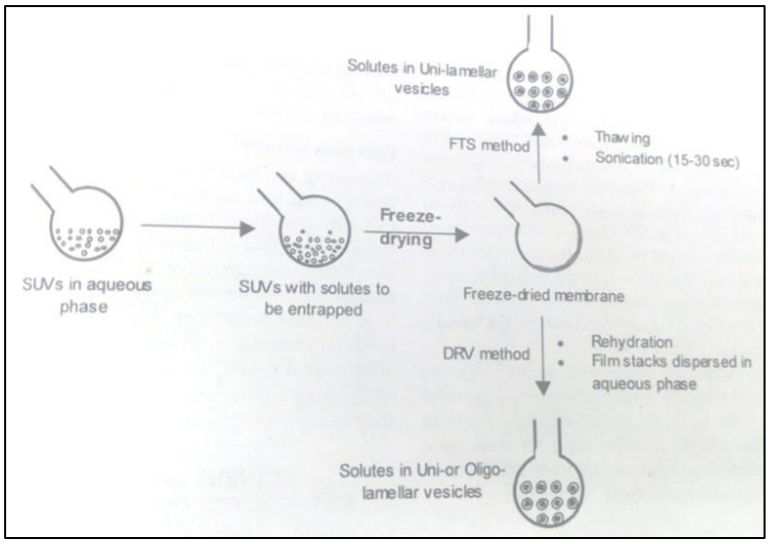


Fig No. 8 : Dried Reconstituted Vesicles.

g . Freeze-Thawed Liposome [2,47]

The FTS method is an addition of traditional DRV method. The method is depend upon freezing of a unilamellar dispersion and then thawing by keeping at room temperature for predetermined time period of 15min and finally introduced to a brief sonication cycle. Therefore the process ruptures and refuses SUVs during which the solute achieve equilibrium within external and internal environment, and the liposomes themselves fuse and enlarge markedly in size. The entrapment volume can be up to 30% of the total volume of the dispersion (10ml/mg phospholipids). This type of fusion is robustly inhibited by increasing the ionic strength of the medium and by rising the phospholipid concentration. Mounir TraõÈkia et al and its co-worker prepared unilamellar vesicle by repetitive freeze-thaw cycles.[38]

Advantage

FTL method is very simple , time consuming and mild for entrapped solutes which lead to a high proportion of large unilamellar vesicles(LUV) formation .

Disadvantage

Encapsulation efficiency by these method is low compared to DRVs.

Neutral liposomes can not be subjected to freezing and thawing method.

B. Solvent Dispersion Methods

By these method, lipids are primarily dissolved in an organic solution , which is then introduced with an aqueous phase containing materials to be entrapped into the liposomes. The lipids arrange themselves at the interface of organic and aqueous phase forming monolayer of phospholipids ,which forms half of the bilayer of the liposome.

a. Ethanol Injection

Ethanolic lipid solution is rapidly injected to an large amount of saline or aqueous buffer medium. The MLVs are instantly formed .The rate of injection is generally optimize to achieve complete addition , so that phospholipid molecules are dispersed uniformly all through the medium. Chiraz Jaafar-Maalej et al prepared hydrophilic and lipophilic drug-loaded liposome by ethanol injection[46].

Advantage

- These method is very easy.
- Sensitive lipids can be process by these method.

Disadvantage

- By these method heterogeneous (30–110 nm) vesicle were formed.
- Liposomes are very dilute , it is difficult to remove residual solvent like ethanol because it forms azeotropic mixture with water.
- There is chances of degragation or inactivation of biolactive active macromolecules in the presence of even low amounts of ethanol.

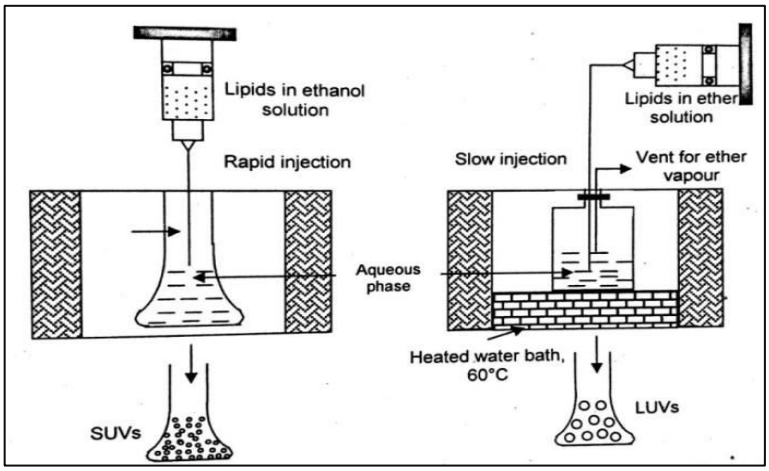


Fig No. 9 : Ethanol And Ether Injection.

b. Ether Injection

In these ether Injection Method solution containing lipids were dissolved in diethyl ether or ether/ methanol mixture which is slowly injected to an aqueous solution of the material to be encapsulated at 55–65°. Following by removal

of ether like residual solvent under vacuum leads to the formation of liposomes. The main disadvantage with the method is liposomes produced are heterogeneous in nature (70–190 nm) and the material to be encapsulated will be exposed to higher temperature which limited the use of thermolabile composition.

c. Double Emulsion Vesicles

In this process, an active ingredient is initially dissolved in an aqueous phase (w1) which is then emulsified in an organic solvent containig polymer to form a primary w1/o emulsion. This primary emulsion is then mixed in an emulsifier which also consist of aqueous solution (w2) to form a w1/o/w2 double emulsion. The extraction of the solvent leaves microspheres in the aqueous external phase, making it possible to seperate them by filtering or centrifuging.

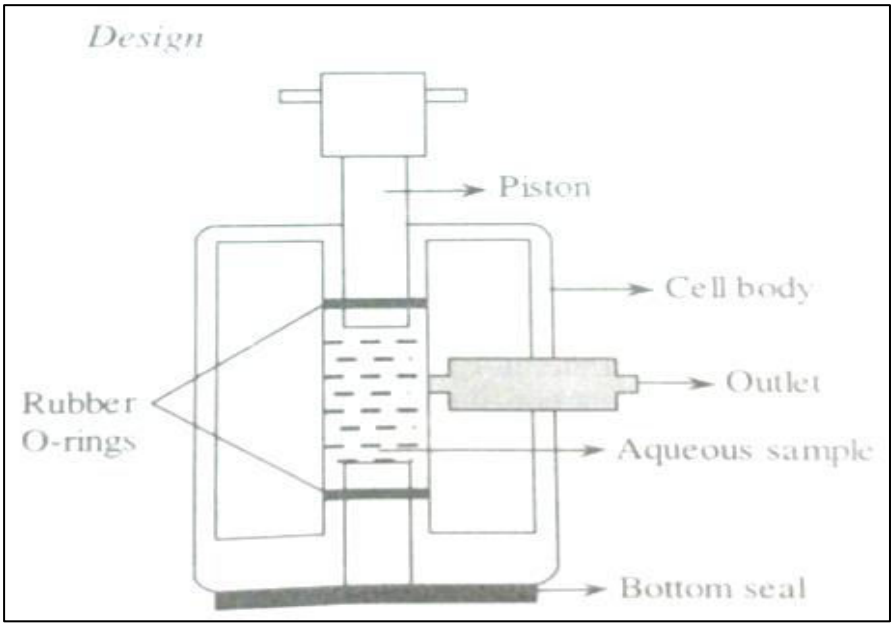


Fig No. 10 : Double Emulsion Vesicles App.

a. Reverse Phase Evaporation Vesicles

W/O emulsion is formed by brief sonication of two phase system containing phospholipids in organic solvent (diethyl ether or isopropyl ether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are seperated under reduced pressure, which turn to form a viscous gel. The liposomes are prepared when residual solvent is separated by continuous rotary evaporation under reduced pressure.Using this method high encapsulation efficiency up to 65% can be achieve in a medium of low ionic strength (0.01M NaCl). These method introduced to encapsulate small and large macromolecules. The main drawback of these method is the exposure of components to be encapsulated to organic solvents which lead to inactivation and to brief periods of sonication. P. Roy Vagelos et al prepared liposome by these method.[37]

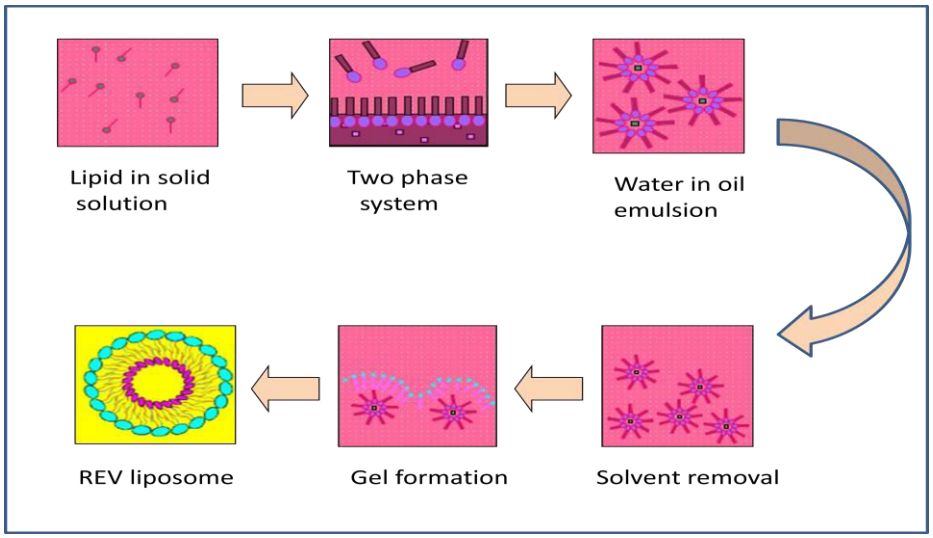


Fig No. 11 : Reverse Phase Evaporation Vesicles.

b. Stable Plurilamellar Vesicles

These method of plurilamellar vesicle preparation followed by formation of water-in-organic phase dispersion with an excess of lipid which further introduce to drying under continued bath sonication with an irregular stream of nitrogen. SPLVs require a large aqueous core, the common of the entrapped aqueous medium being located in the compartment in between adjacent lamellae. The percent entrapment normally ranges around 30%.

C.**Detergent Removal Method**[48,50]

The detergents at their critical micelle concentrations (CMC) also helps in solubilising lipids. As detergent is separated the micelles it will lead to become progressively rich in phospholipid and ultimately combine to form LUVs. The detergents also can be removed using dialysis. These method have advantages like excellent reproducibility and formation of liposomes which has uniformity in size. The main disadvantage of the method is the incorporation of detergent in minute quantity within the liposomes. A commercial device called LIPOPREP which is a version of dialysis system existing for the removal of detergents. Other techniques have been used for the removal of detergents:

a. Column Chromatography

Phospholipids in the form of either sonicated vesicles or as a dry film , at a molar ratio of 2:1 with deoxycholate form unilamellar vesicles of 100nm. Deoxycholate remove using column chromatography .This could be done by the passing the dispersion over a Sephadex G-259 column presaturated by constitutive lipids and preequilibrated using hydrating buffer.

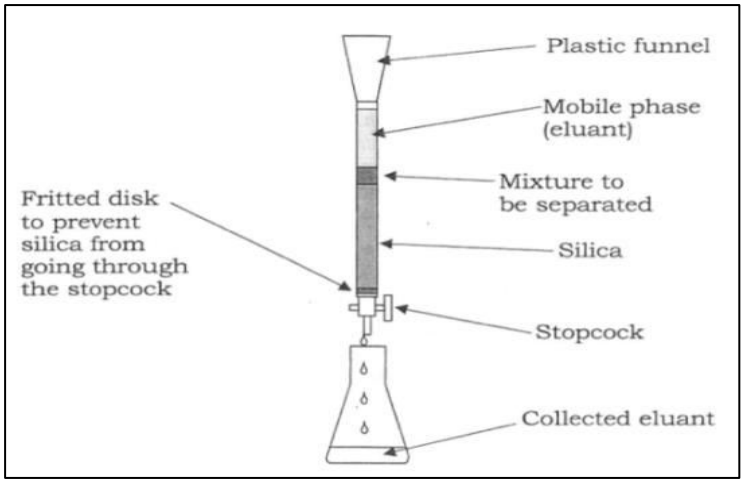


Fig No . 12: Column Chromatography.

a. By Detergent Adsorption Using Bio-Beads

Detergent (non-ionic)/phospholipid mixture which may form large unilamellar vesicles (LUV) upon removal of Triton X-100 (non-ionic detergent) by appropriate adsorbents for detergent. On addition of aqueous phase on the casted lipid film with 0.5-1.0% Trioton X-100, washed Bio-beads are added to dispersion (0.3g wet bio-beads/ml of dispersion) and rocked for about 2h at 4 ± 1^0C.

b. Dialysis[49]

Detergent are mainly soluble in both aqueous as well as organic media and there is an equilibrium within the detergent molecules in the water phase, and in the lipid environment of the micelle. The CMC can give an indication to the position of this equilibrium. Upon reducing the concentration of detergent in the whole aqueous phase, the molecules of detergent can be washed away from mixed micelle by dialysis. The action of egg PC with a 2:1 molar ratio of sodium cholate followed by dialysis which lead to the formation of vesicles (100nm). A commercial version of the dialysis system is available under the trade name LIPOPREP™ (Diachema AG, Switzerland).

2. Active Loading[2]

The exploitation of liposomes as drug delivery system is encouraged with the advancement of well-organized encapsulation procedures. The membrane from the lipid bilayer is in general impermeable to ions and larger hydrophilic molecules. Ions transport can be synchronized by the ionophores though permeation of neutral and weakly hydrophobic molecule can be inhibited by concentration gradients. A few weak acid or bases yet, can be transported throughout the membrane because of various transmembrane gradient, such as electric, ionic (pH) or specific salt (chemical potential) gradient. Some method exist for improved incorporation of of drugs, including remote (active) loading method which load drug molecules into preformed liposome using pH gradient and potential

difference across liposomal membrane. A concentration variation in proton concentration across the membrane of liposomes can drive the loading of amphipathic molecule.

Active loading methods have the following benefit over passive encapsulation Technique[2, 49]

- It will lead to high encapsulation efficiency and capacity.
- Using these method leakage of the encapsulated compounds can be reduced.
- "Bed side" loading of drugs therefore limiting loss of retention of drugs by diffusion, or chemical degradation while storage.
- These process is Flexibile for constitutive lipid, as drug is loaded after the formation of carrier unit.
- It also reduce the safety hazard by avoiding biologically active compounds in the preparation step during dispersion.
- The transmembrane pH gradient may be occured by various method. Based upon the nature of drug to be encapsulated.

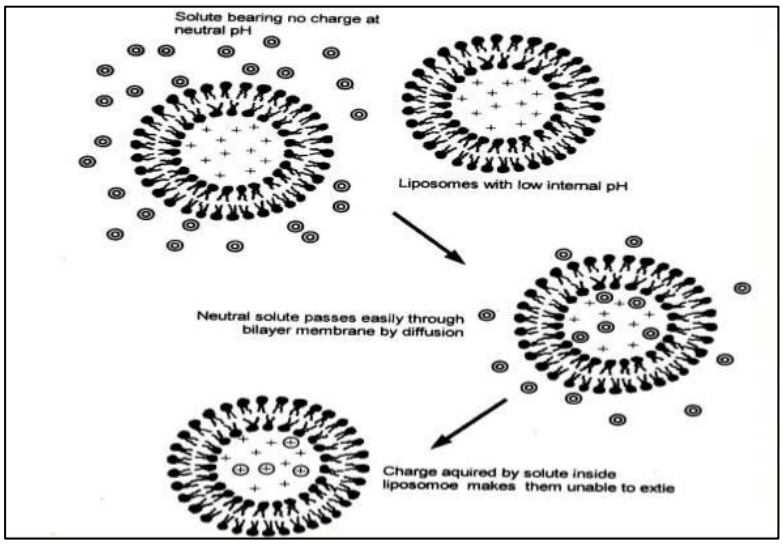


Fig no. 13 : Active Loading.

Characterization Of Liposomes[2]

The behaviour of liposomes in both physical and biological systems in governed by the factors such as:

- ❖ Physical size
- Membrane permeability
- Percent entrapped solutes
- Chemical composition
- Quantity and purity of the starting material

Therefore, the liposomes are characterized for physical attributes:

- ❖ Shape size and its distribution
- Percentage drug capture
- Entrapped volume
- Lamellarity
- Percentage drug release
- ❖ Chemical compositions:
- Estimation of phospholipids
- Phospholipid oxidation
- Analysis of cholesterol

Drug Release:

Drug release mechanism from the liposomes can be assessed by the use of a well calibrated in vitro diffusion cell. The liposome based formulations with the help of in vitro assays can predict pharmacokinetics and bioavailability of the drug previous to employing costly and time consuming in vivo studies. The dilution-induced drug release in buffer and plasma was working as predictor for pharmacokinetic concert of liposomal formulations and an additional assay which determined intracellular drug release induced by liposome degradation in the presence of mouse-liver lysosome lysate was used to measure the bioavailability of the drug.

Mechanism of Transportation Through Liposomes[42]

Liposomes can interact with cells by four type of mechanisms

• Endocytosis by phagocytic cells of the reticuloendothelial system RES such as macrophages and neutrophils.

• Adsorption to the cell surface either by nonspecific weak hydrophobic or electrostatic forces or by specific communications with cell-surface constituents.

• Fusion with the plasma cell membrane by introduction of the lipid bilayer of the liposome into the plasma membrane, with instantaneous release of liposomal composition into the cytoplasm.

• Transport of liposomal lipids to cellular or subcellular membranes, or vice versa, lacking any association of the liposome contents. It often is hard to conclude what mechanism is functioning and more than one may work at the same time.

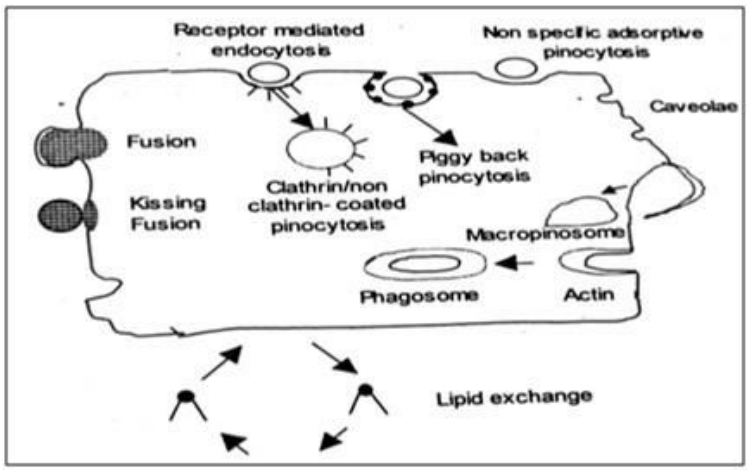


Fig No. 14 : Mechanism Of Transportation

Application of Liposome[53]

1.Liposome as drug/protein delivery vehicles

- Controlled and sustained drug release
- Enhanced drug solubilization
- Altered pharmacokinetics and biodistribution
- Enzyme replacement therapy and biodistribution
- Enzyme replacement therapy and lysosomal storage disorders

2. Liposome in antimicrobial, antifungal and antiviral therapy

- Liposmal drugs
- Liposomal biological response modifiers

3. Liposome in tumour therapy

- Carrier of small cytotoxic molecules
- Vehicle for macromolecules as cytokines or genes

4. Liposome in gene delivery

- Gene and antisense therapy
- Genetic (DNA) vaccination

5. Liposome in immunology

- Immunoadjuvant
- Immunomodulator Immunodiagnosis

6. Liposome as artificial blood surrogates

7. Liposome as radiopharmaceutical and radio diagnostic carriers

8. Liposome in cosmetics and dermatology

9. Liposome in enzyme immobilization and bioreactor technology.

Stability of Liposomes[54]

Throughout the improvement of liposomal drug products, the stability of the processed formulation is of main concerned. The therapeutic activity of the drug is governed from the stability of the liposomes from the manufacturing steps to storage to delivery. A stable dosage forms is recognised by the physical stability and chemical reliability of the active molecule throughout its developmental process and storage. A well premeditated stability study includes the assessment of its physical, chemical and microbial parameters in conjunction with the declaration of product's integrity during its storage period. Therefore a stability protocol is crucial to study the physical and chemical reliability of the drug product in its storage. The official stability study criteria for liposome is shown in fig no .15.

A. Physical Stability

Liposomes are bilayered vesicles that are produced when phospholipids are hydrated in water. The vesicles obtained through this procedure are of dissimilar sizes. For the period of its storage, the vesicles lead to aggregate and enlarge in size to reach thermodynamically favorable state. During storage, drug leakage from the vesicles can take place owing to fusion and infringement of vesicles, which deteriorates the physical stability of the liposomal drug product. Consequently morphology, size and size distribution of the vesicles are significant parameters to consider the physical stability. With the aim of monitor this, a selection of techniques like light scattering and electron microscopy can be used to assess the visual appearance (morphology) and size of the vesicles.

B. Chemical Stability

Phospholipids are chemically unsaturated fatty acids that are tending to oxidation and hydrolysis, which may change the stability of the drug product. In conjunction with this, pH, ionic strength, solvent system and buffered species also perform a key role in controlling a liposomal formulation. Certain chemical reaction can be take place still by light, oxygen, temperature and heavy metal ions. Oxidation deterioration involves the development of cyclic peroxides and hydroxyperoxidases because of the effect of free radical creation in the oxidation progression. Liposomes can be prohibited from oxidative degradation by protecting them by light, by adding up anti-oxidants such as

alphatocopherol or butylated hydroxyl toluene (BHT), producing the product in an inert environment (presence of nitrogen or Argon) or by adding EDTA to eliminate trace heavy metals. Hydrolysis of the ester bond at carbon position of the glycerol moiety of phospholipids turn into the formation of lyso-phosphatidylcholine (lyso PC), which increase the permeability of the liposomal contents. Consequently, it becomes essential to control the limit of lysoPC inside the liposomal drug product. This can be processed by formulating liposomes using phosphatidylcholine free from lyso PC .

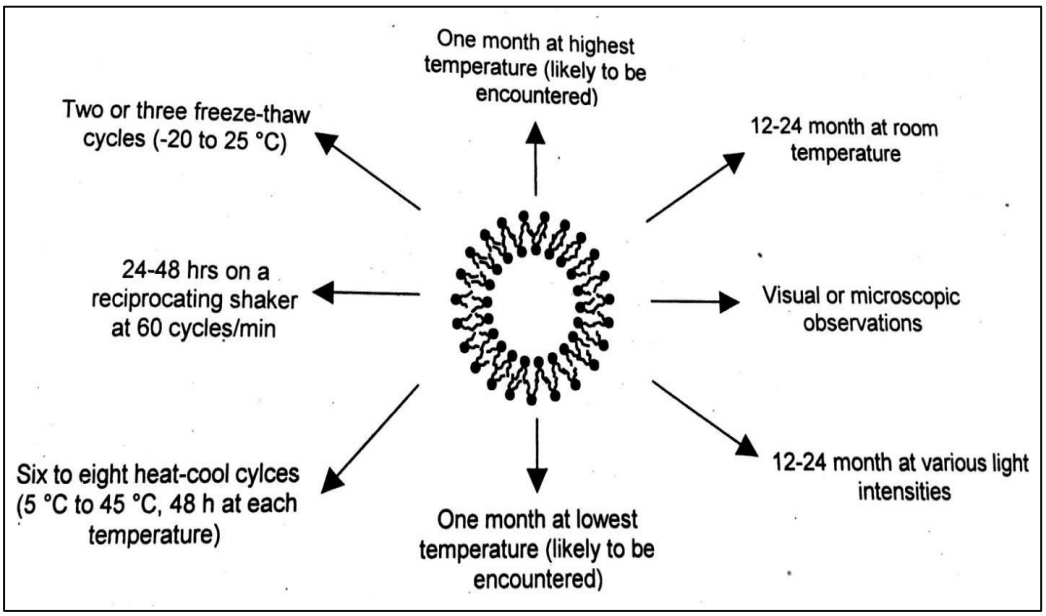


Fig No. 15 : Stability of Liposomes.

Marketed Products [5,54,57]

Table No. 5 : List of Marketed Products

Marketed product	Drug used	Target diseases	Company
DoxilTM or CaelyxTM	Doxorubicin	Kaposi‟s sarcoma	SEQUUS,USA
DaunoXomeTM	DaunSolid tumoursorubicin	Kaposi‟s sarcoma, breast & lung cancer	NeXstar, USA
AmphotecTM	Amphotericin-B	fungal infections, Leishmaniasis	SEQUUS, USA
Fungizone®	Amphotericin-B	fungal infections, Leishmaniasis	Bristol-squibb, Netherland
VENTUSTM	Prostaglandin-E1	Systemic	The liposome

		inflammatory diseases	company, USA
ALECTM	Dry protein free powder of DPPC-PG	Expanding lung diseases in babies	Britannia Pharm, UK
Depocyt	Cytarabine	Cancer therapy	Skye Pharm, USA
Topex-Br	Terbutaline sulphate	Asthma	Ozone, USA
Novasome®	Smallpox vaccine	Smallpox	Novavax, USA
Avian retrovirus vaccine	Killed avian retrovirus	Chicken pox	Vineland lab, USA
Doxil®	Doxorubicin Hcl	Refractory ovarian cancer	ALZA, USA

Table No. 6 : Some liposomal cosmetic formulations currently on the market.

Product	**Manufacturer**	**Liposomes and key ingredients**
Efect du Soleil	L‟Or´eal	Tanning agents in liposomes
Niosomes Lancome	(L‟Or´eal)	Glyceropolyether with moisturizers
Nactosomes Lancome	Lancome (L‟Or´eal)	Vitamins
Formule Liposome Gel	Payot (Ferdinand Muehlens)	Thymoxin, hyaluronic acid
Future Perfect Skin Gel	Estee Lauder	TMF vitamins E, A palmitate, cerebroside ceramide, phospholipid
Symphatic 2000	Biopharm GmbH	Thymus extract vitamin A palmitate
Natipide II	Nattermann PL	Liposomal gel for do-it-yourself
Flawless finish	Elizabeth Arden	Liquid make-up
Inovita	Pharm/Apotheke	Thymus extract, hyaluronic
Eye Perfector	Avon Soothing	Niosomes, , cream to reduce eye

Conclusion

Several drug candidates which are extremely potent and have low therapeutic indication can be targeted to the vital diseased site by means of the liposomal drug delivery system. Drugs encapsulated in liposomes can cover a significantly distorted pharmacokinetics. The efficiency of the liposomal formulation based on its capability to transport the drug molecule to the targeted site over a long-lasting period, concurrently dropping its (drug's) toxic effects. The drugs are encapsulated inside the phospholipid bilayers and are ordinary to diffuse out from the bilayer gradually. A variety of factors like drug concentration, drug to lipid ratio, encapsulation efficiency and in vivo drug release must be considered during the formulation of liposomal drug delivery systems. The development of deformable liposomes and ethosomes in conjunction with the administration of drug loaded liposomes during inhalation and ocular route are some of the advances in the technology. Therefore liposomal approach can be successfully utilized to progress the pharmacokinetics and therapeutic efficacy, concurrently falling the toxicity of various highly potent drugs.

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