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Review on Liposomes: A Novel Carrier for Bioactive Compounds

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ABSTRACT

Liposomes were discovered by Alec D Bangham in the year 1960.The term liposome means lipid body. They are categorized as a drug delivery system in which drug is targeted to a particular tissue. Liposomes can range from 25nm to 500nm in size. Usually, they have a sphere shape vesicle. They may be having one phospholipid bi- layer or more. They are widely used as drug targeting delivery system because they can penetrate tissues more precisely than free drugs and they have similarity between their lipid bilayer and cell membrane. They are also preferred more because of their drug entrapment efficiency. Liposomes are biocompatible i.e. they are capable to entrap both hydrophilic and lipophilic drug hence they solve the problem of choosing a suitable outer covering layer for the formulation as, they are suitable for both hydrophilic and lipophilic drug molecules. Liposomes are the modern world technology to deliver the drugs to a specific targeted organs or tissue. There exist a lot of methods for preparation of liposomes and they are classified according to their structure and methods of preparation which are discussed in brief in this review article, along with liposome advantages and disadvantages, handling of liposomes, evaluation parameters and liposome applications.

Keywords: *Liposomes, Drug delivery system, Drug entrapment, Biocompatible.*

INTRODUCTION

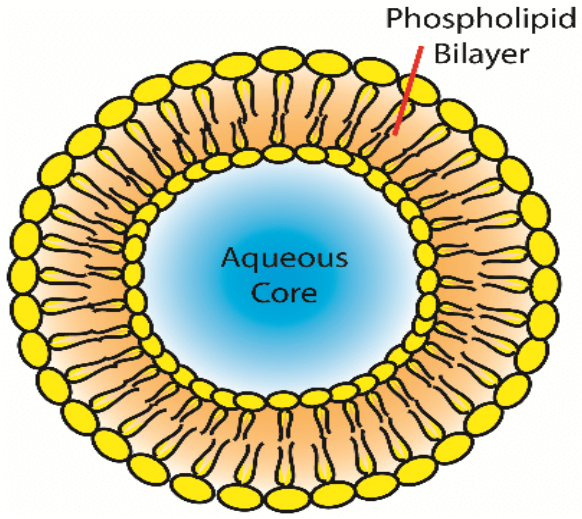
In the year 1906, Paul Ehrlich started working on targeted drug delivery. He named it as magic bullets[1-2] later in the year 1961 A.D. Bangham along with his colleagues worked in this field and got great success. The word liposome is extracted out of 2 Greek word's "lipos" which represents fat and "soma" which represents body as a whole liposome means fat body. Liposomes are colloidal vesicular structure, composed of two layers of fat membrane hence they are called as bilayers. In a liposome drug is entrapped in an aqueous media in the inner layer of liposome. Drug that is entrapped in the liposome will get extension in its therapeutic shelf life [3-4].

In today's world liposomes are mainly used in cosmetic industries and pharmaceutical industries. As liposomes have special ability to entrap unstable molecules inside its core layer, they are also used in agriculture and food industries. As they need to preserve a lot of unstable molecules such as flavours, fragrance, antioxidants, bioactive compounds, etc. Liposomes not only entrap unstable molecules but also shield their functioning. Liposomes have potency to trap both hydrophilic and hydrophobic compounds and also prevent their decomposition.[5] As liposomes are good drug targeting delivery system, biocompatible, less toxic, biodegradable and highly efficient in drug entrapment they are gaining a vast hike in their demand as investigational tool in research and as an commercial drug target delivery system. Liposomes are also used in cancer/tumor therapy, gene therapy, immune modulation, skin and topical care cosmetic products, etc.

Liposome structure

Liposome structure is like spherical vesicles, into which a liquid medium or aqueous phase is entrapped along with drugs into a thin lipid bilayer. This thin bilayer is composed of phospholipids. In this phospholipid molecule it constitutes a head group that is attracted towards water and a tail group which is repelled by water [6].

In natural environment phospholipids are found as bilayer, so are liposomes. Inside a cell one hydrophilic head group faces towards environment water outside the cell and inner vesicle hydrophilic head groups faces towards water of aqueous phase which is entrapped in it. Both layers hydrophobic tail groups face each other and together they composed bilayer structure of liposome. When a liposome layer breaks in to small fragments and they form a monolayer molecule it is called as "micelles".



Structure of Liposome

Advantages of liposomes [8]

1) Liposomes are completely environment friendly as they are biodegradable.
2) Liposomes are non- toxic as they do not accumulate in body and cause toxicity.
3) Liposomes are biocompatible and immunogenic.
4) Liposomes are suitable for drugs which are hydrophilic, hydrophobic and amphiphatic.
5) Liposomes increases stability of drug which are encapsulated inside it.
6) Liposomes make sure to reduce exposure of toxic drugs which are encapsulated in it to sensitive tissues.
7) Liposomes encapsulation promotes potency and therapeutic properties of drug encapsulated inside it.
8) Liposome promotes pharmacokinetic effects such as, reduction in elimination time, increasing circulation life time.
9) Liposomes provide a god way of selective passive targeting for tumor tissues.
10) Liposomes are a preferred method to produce drugs with controlled and sustained release dosage form.

Disadvantages of liposomes [9-10]

1) Liposomes production is expensive.
2) There is slight possibility of leakage of encapsulated drug.
3) There is possibility of fusion of encapsulated drug with liposome membrane.

4) When liposomes are given via IV route, it will be quickly excreted out from our blood by kupfer cells and reticulo endothelial system.
5) Liposomes has shorter biological half life.
6) Phospholipids of liposomes may get oxidized or hydrolyzed.
7) Liposomes sometimes may face stability issues.
8) Liposomes have low solubility problem.

Classification of liposomes [11-14]

Liposomes are categorised on two basis, they are:

1. Based on their structure/lamella-

They are of two types:

A. Uni-lamellar vesicles: they are also called as ULV. They are made up of single layer of phospholipids which encloses their aqueous solutions. They are of four types.

- Small unilamellar vesicles- also called as SUV. Its size ranges from 20nm-40nm.
- Medium unilamellar vesicles- also called as MUV. Its size ranges from 40nm-100nm.
- Large unilamellar vesicles- also called as LUV. Its size ranges from 100nm-1000nm.
- Giant unilamellar vesicles- also called as GUV. Its size ranges from 1000nm and above.

B. Multi-lamellar vesicles: they are also called as MLV. They are made up of multiple layers of phospholipids which enclose their aqueous solutions. Their arrangement looks like onion arrangement.

2. On the basis of their Method of Preparations-

There are of six types:

- ULV-REV: They are ULV type of liposomes created through reverse phase evaporation method.
- MLV-REV: They are MLV type of liposomes created through reverse phase evaporation method.
- SPLV: They are liposomes created by stable plurilamellar vesicles.
- FAT-MLV: They are MLV type of liposomes created by frozen and thawed method.
- VET: They are liposomes created by vesicles extrusion technique.
- DRV: They are liposomes created by dehydration-rehydration vesicles technique.

Methods of Preparation of Liposomes

Generally, there are four steps to make a liposome, they are:

- Obtained lipid from organic solvent needs to be dried out.
- Making solution of obtained lipids along with aqueous media.
- Purify the prepared liposome.
- Analyses of the finally prepared liposome.

To prepare a good liposome we must follow certain parameters, some of them are as follows [15-16]:

- Nature of the lipid.
- Nature of the drug entrapped.
- Nature of the medium in which drug is dissolved.
- Concentration of the entrapped drug in the liposome.
- Toxicity of drug in the liposome.
- Optimum size of the lipid particles.

There are two categories of liposome preparations:
1. Preparations by using general methods.
2. Preparations by using specific methods.

I. Preparations by using general methods: In this type of method obtained lipid is mixed in an organic solvent. Now newly created lipid solution is evaporated (heated) and some thin films of lipid is left near container walls. Now aqueous solution of drug is mixed with the lipid layer. Now there goes two processes. In first process the aqueous phase is agitated and multi lamellar vesicle is created which after sonication results into SUV's. In the second process the aqueous phase is sonicated first and then evaporated and gets LUV's as result.

Drugs which can be dissolved in water it can be incorporated into aqueous solution or drugs which can't be dissolved in water it can be incorporated into organic solvent.

II. Specific methods of preparations: on the modes of dispersion there are three types of specific methods for liposome preparation. They are:

- Physical dispersion methods.
- Solvent dispersion methods.
- Detergent solubilization methods.

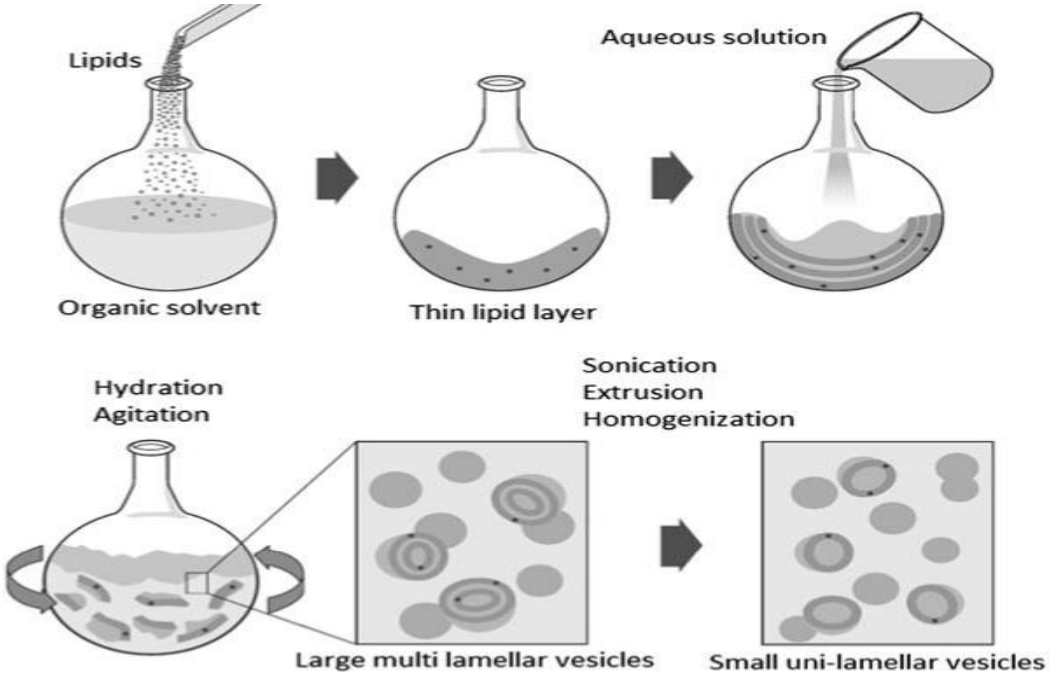
A. Physical dispersion methods: In this type of methods water-soluble drug is wasted in a large quantity as they have only 5%-10% of capacity of water-soluble drug entrapment, where as they have high capacity to enclose lipid soluble drugs. There are many types of physical dispersion methods, some of them are:

- Lipid film hydration by hand shaking and non hand shaking method.
- Freez thawed method.
- Sonication method.
- French pressure cell method.
- Membrane extrusion method.

a. Lipid film hydration by hand shaking and non hand shaking method:
In this method, lipid layers are formed from their organic solutions under reduced pressure by using flash rotatory evaporator. We can also try hand shaking process next to dispersion of lipid films into aqueous solution. After these steps lipid layers swell up and sticks to round bottom flask wall's and vesiculate into MLV's.

In hand shaking method manual agitation creates mechanical energy while in non hand shaking method energy is provided by exposing films to a stream of water and saturated nitrogen for 12-15 minutes.

By this method we can get drug entrapment capacity of 30%.



b. Freez thawed method [17-18]:

In this method lipid's before introduction into aqueous media is freezed into a finely divided form. Solvent used in this method is tertiary butanol.

By this method we can get drug entrapment capacity of 20%-30%.

c. Sonication [19]:

It is a widely used method to prepare SUV's liposomes. In this method there is reduction of vesicles which imparts energy to produce liposomes. In sonication ultrasonic energy is imparted on MLV liposomes which get's reduced and converted into SUV's.

There are two types of sonication methods, they are as follows:

1. Bath sonicator.
2. Probe sonicator

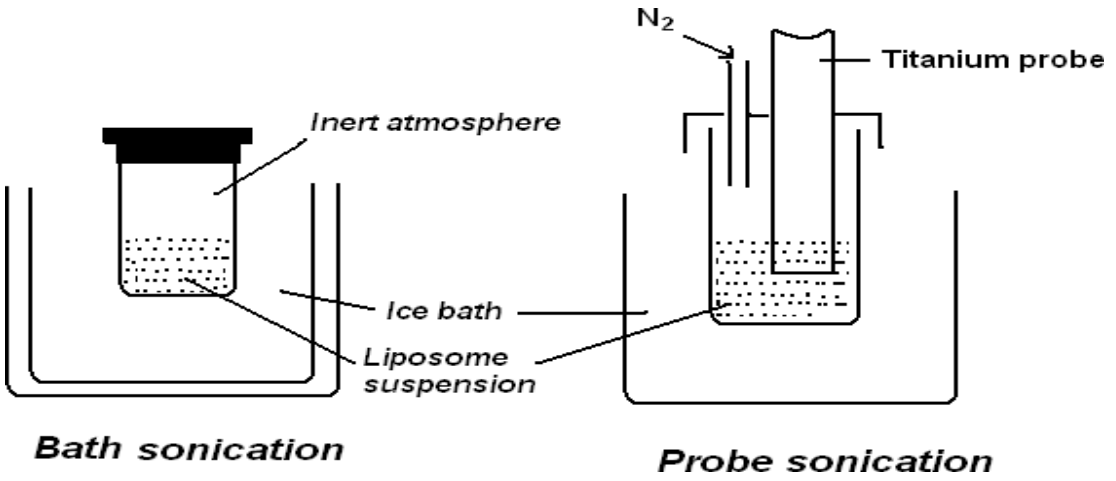
Bath sonicator is useful with large quantity of lipids. Whereas probe sonicator is useful with small quantity of lipids with very high energy.

Probe sonicator's main disadvantage is contamination of prepared liposomes by the metal tip of the probe.

Although this is widely accepted method but still it has many disadvantages such as:

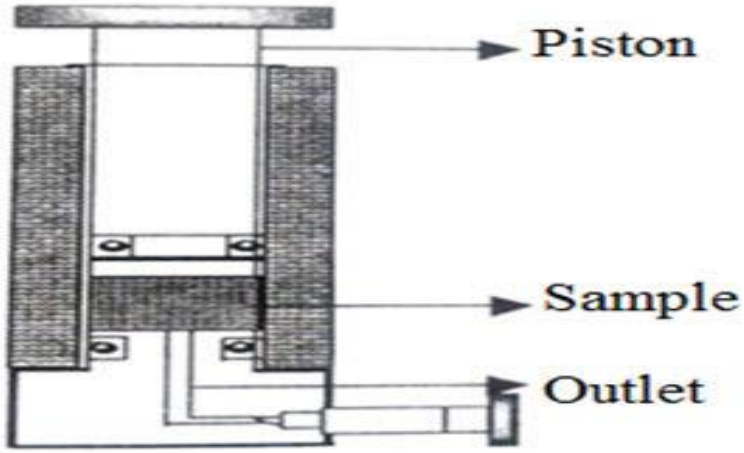
- Phospholipids degradation
- It contains some MLV's and SUV's.
- Metal pollution from probe tip.
- Low drug entrapment efficiency.

By this method we can get drug entrapment capacity of 30%.



d. French pressure cell method [20]

In this method under highly applied pressure through a small orifice uni or oligo liposomes of size range of 30nm-80nm are extracted out. By this method dispersed MLV's can be transformed into SUV's. This method operates at 2000 psi at 45^{o}c temperature. This method has many merit points as compared to sonication method such as it is a simple and rapid method. This method involves with gentle handling of unstable materials along with high reproducibility. As compared to sonicated liposomes french pressure cell SUV's liposomes are larger in size. Main drawback of this method is it is hard to maintain such a high temperature and its working volume is also very small about 50ml.



French Pressure Cell Apparatus

e. Membrane Extrusion Method

In this method liposome preparation is permitted to flow via a membranous filter having fixed pore size to decrease the molecular size of the liposome. This method is mainly applied on LUV's and MLV's. to execute this method special equipment is setup which involves a pump that pushes down the liposome suspension through the membrane to get desired size of liposome. There are two types of membranes used in this method they are the tortuous path type and nucleation track type. There are various factors on which this method depends such as applies pressure, pore size of membrane, diameter of liposome particles, and number of

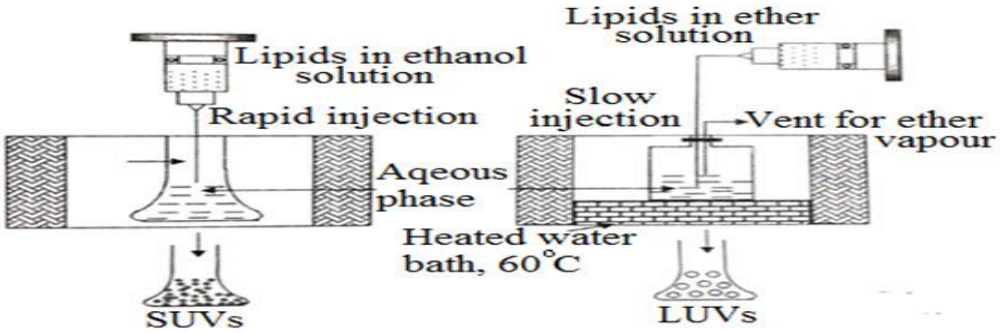
cycles. In this method there is no degradation of phospholipids, this method is easy to use, highly reproducible along with high drug entrapment capacity.

B. Solvent Dispersion Methods: During this method, initially lipids need to be dissolved into an organic solvent then it is mixed with the aqueous phase containing drug to be entrapped into the lipid bilayer. When organic solvent and aqueous phase meets at its interface phospholipids align themselves and forms monolayer and then forms bilayer. This method is of two types, they are:

1) Ethanol injection method
2) Ether injection method

1) Ethanol injection method [21]: During this method a solution containing lipids and ethanol is taken into a injection and with the help of a fine needle it is ejected rapidly into a aqueous medium or excess of saline solution. As ethanol dilutes into H_2O all the phospholipid molecules are scattered equally into the medium. By this method high yield of SUV's of 25nm diameter are produced. As it is a simple process it contains some disadvantages such as the resultant liposome are heterogeneous, liposomes are less concentrated, it is very hard to extract out all ethanol molecules because it forms azeotrope with H_2O.

2) Ether injection method [22-24]: During this method, either diethyl ether or ether/methanol mixture is mixed with solution of lipids and is patiently injected into aqueous solution of material to be encapsulated with the help of injection at 55^oc-65^oc. Under reduced pressure. During this method, lipids are operated carefully as result there is chances of oxidative degradation. Main drawback of this method is it is a long and time-consuming method also there is requirement of controlled and precise introduction of lipids into solution, also we get heterogeneous liposomes as result. Also, we need high temperature. Liposome drug entrapment efficiency is low but drug entrapment capacity is high.



ETHER INJECTION **ETHANOL INJECTION**

C. Detergent Solubilization Methods: During this method, lipids come closer to aqueous solution via detergents. With the interaction of lipid- detergent-aqueous solution a structure is formed called as micelles. The concentration of detergent in an aqueous medium from where micelles start to form is known as critical micelle concentration. Without critical micelle concentration detergent exist in free state in aqueous medium. As we get CMC state then on increasing detergent concentration we get more micelles, at a point we get to know on increasing concentration of detergent we get reduction in micelles size. Now when the detergent is removed, we get our LUV's liposomes. To remove detergents there are many methods like dialysis, dilution, gel-permeation

chromatography, etc. but dialysis is widely used method to remove detergents as they have many advantages over other methods like they have excellent reproducibility and they produce large size population of homogeneous liposomes. Only one disadvantage in this method is that there is retention of detergents in the liposomes.

Liposome loading [25]

Drug loading could be of two types, either passively which means encapsulated substance is entrapped when liposome is getting created or by active means which means encapsulated substance is entrapped after liposome is formed. Some hydrophobic drugs like amphotericin B taxol are loaded with passive method. Drug entrapment in passive loading is 100% but it also varies on drug's solubility in liposome membrane.

Benefits of drug loading in liposomes:

- Targeting of drug is accurate
- Drug wastage is less
- Sustained release system.
- Promotes solubility of lipophilic as well as amphiphilic drug.
- Improves drug penetration.

Evaluation of liposomes [26-30]

As our liposome is created they needs to be evaluated to predict they are in vitro type or in vivo type. There are three parameters for evaluation they are:

1) Physical parameters: It involves surface features, size, shape, lamerallity and drug release profile.
2) Chemical parameters: It includes informations which include quality and capablity of liposomal constitutes.
3) Biological parameters: it includes informations regarding safety and compatibility for therapeutic use of liposome.

Some types of categories for evaluation of liposomes:

1) Vesicle shape: It can be determined by electron microscope.
2) Lamellarity: it is number of bilayers present in liposome and is determined with freeze fracture electron microscopy.
3) Surface charge: it is determined using Zeta potential and free flow electrophoresis techniques.
4) Encapsulation efficiency: It shows quantity of water dispersible drugs in aqueous solution of liposome.
5) Drug release: Drug release can be tracked by using well calibrated in vitro diffusion cell.

Purification of liposomes [31-32]

Main method for liposome purification is chromatography, dialysis, gel filtration and centrifugation. Sephadex 50 in chromatographic purification is widely used. Hollow fiber dialysis cartridge is used in dialysis purification. In centrifugation purification for SUV's 200000 g for 10-20 hours. For MLV's 100000 g for less than one hour.

Application of liposomes [33]

1) It is used in gene therapy.
2) It is used in leishmaniasis.
3) It is used in cosmetics.

4) It is used in dermatology.
5) It is used in enzyme immobilization.
6) It is used as carriers for vaccines.
7) It is used in topical use.
8) It is used in radio- diagnosis.
9) It is used to safeguard entrapped drug from enzymatic degradation.
10) It enhances anti microbial safety.

CONCLUSION

Liposomes in modern world play a very tremendous role as a targeted drug delivery system as well as a useful investigation tool for research work. Liposomes have a very flexible behavior as they are excellent drug delivery system they do not depend on route of administration and also, they are not varied on drug or materials physiochemical properties. Being a low toxicity drug delivery system, they gain a lot of fame in recent times. With such a good response in recent times it will be very easy to predict that liposomes will have a very good and bright future in upcoming time.

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