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Liposomes: A Comprehensive Review on Development & Evaluation Parameters

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ABSTRACT

Liposomes were the first nanomedicine to receive authorisation for clinical use. Liposomes' remarkable ability to stop drug deterioration and reduce undesirable side effects has led to an increase in their application for targeted drug administration. Drugs in liposomes can either be incorporated into the phospholipid bilayer (hydrophobic drugs) or the aqueous space (hydrophilic drugs). The primary topics explored in this study are liposome classification, liposome production, utilises and their benefits and limitations. Nowadays, liposomes have captured the keen interest of most researchers. Because they are uncomplicated to create, effective against a particular ailment, and offer a number of extra advantages over others. This review discussed the current state, limitations, and uses of liposomes as a modified dosage form. Because liposomes work well as therapeutic agents, many formulations based on liposomes with increased drug concentrations have been created

Keywords: *Liposomes, Polymer, Targeted Drug Delivery, Controlled release*

1. INTRODUCTION

Liposomes possess vesicular structures, and are colloids, with lipid bilayers and with aqueous compartments. Different varieties of the drugs can be delivered by the means of liposomes like antifungals, anticancer, peptides, protein, hormones, enzymes and antimicrobial agents. After releasing drug in blood stream, sustain level of the drug can be maintained by the means of liposomes. The main ingredients of liposomes are cholesterol and phospholipids, the final product is small spherical vesicles. Because of their lipophilic as well as hydrophilic nature, they are attractive delivery system for the different researchers. & are biological compatible ahead of the metabolism and excretion produce sustained effect.[1,2]

Various properties like manufacturing process, size, charge of the surface and composition of lipid, plays an important role in the property of the liposomes.

Lessen toxicity while improving effectiveness, safety and the ability to focus on specific sites are the primary goals of a delivery system.[2]

Mainly liposomes are phospholipid bilayer structure and effectively synthesised of natural & synthetic phospholipid. Alec D. Bangham and colleagues made the initial discovery of the liposome's structure in 1961.This word comes from lipo meaning fatty and soma means structure and their size run from 25 to 500nm.[3]

The purpose of the liposomes' formation is to carry drugs to specific sites, increase circulation, lengthen the medication's duration of action, and release the drug gradually while protecting it from enzymes that break down the drug.[4]

The liposome transports the medication directly to the designated site of action, boosting therapeutic efficacy, assisting in the prevention of drug degradation, shielding the body from improper disposal and unfavourable drug reaction.[5]

Liposomes offers a targeted delivery for a variety of medications, including antifungal, anticancer, antidepressant, and

asthmatic medications. They also assist in delivering the active chemical as a pharmacological entity at a specific location.[6]

Phospholipids have an amphipathic structure, which makes it easier to combine lipid- and water-soluble medications. The hydrophilic head gets repelled by the lipid, whereas the lipophilic tail gets repelled by water. In the same structure, the one part of the liposomes faces the outer portion of the cell and secondly, the other part faces towards the interior portion of liposomes. The bilayer structure of liposomes originates from the natural sources having biologically inert, immunogenic and also exhibit a low toxicity.[6,7]

Advantages of Liposomes[8,9,10]:

1. It is able to transport medications that are soluble/miscible in water &lipids.
2. Allows delivery of drugs directly to cancerous tissues in a passive manner.
3. Liposomes (actinomycin-D) increased therapeutic index and effectiveness.
4. Encapsulation contributed to enhancement in liposome's stability.
5. Don’t causes immune responses.
6. The toxicity of the substance that is encapsulated is decreased by the use of liposomes (amphotericin B, Taxol).
7. The use of liposomes may assist limit the amount of hazardous medication that is taken into sensitive tissues.
8. The capability to pair with site-specific ligands, which is necessary for active targeting.
9. Increased effectiveness in terms of pharmacokinetics
10. It can incorporate both small and large molecules.
11. In addition to this, it serves as a narcotics storage facility.
12. Liposomes have the ability to alter the way drugs are distributed in the body.

Disadvantages of Liposomes[8,9,10]:

1. A low degree of soluble.
2. Phospholipids will sometimes experience an oxidation and a process that is similar to hydrolysis.
3. A rather short half-life.
4. The possibility of the encapsulated substance or molecules leaking out or fusing together.
5. The cost of production is rather expensive.
6. Allergic reaction may be triggered by liposomal components.
7. Because of their huge size, they provide a challenge when targeting certain tissues.

STRUCTURAL COMPONENTS OF LIPOSOMES [11,12]

1. Phospholipids.
2. Cholesterol.

1. Phospholipid

The primary constituents of biological membranes are phospholipids. Generally, phospholipid made up of two fatty acids in which one is phosphate group and other is glycerol molecule.

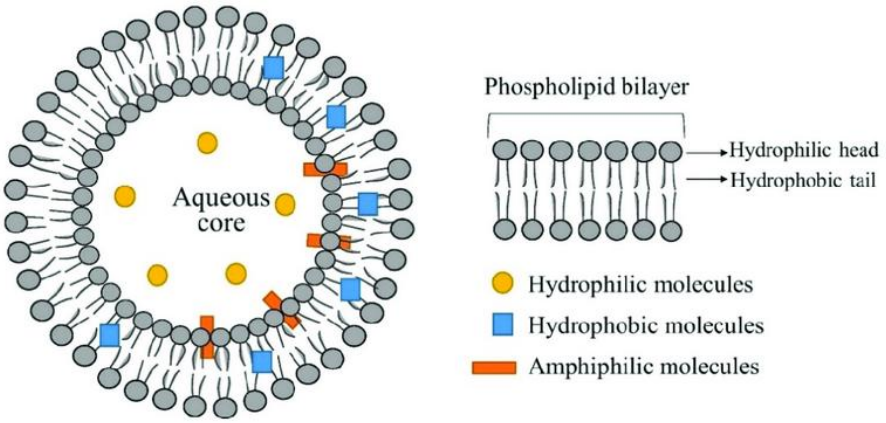


Figure 1. Schematic representation of liposomes.

Phospholipid consists of two parts.

1. Head
2. Tail

Head is hydrophilic part which consist of three molecular components arephosphate, choline & glycerol. Tail is hydrophobic part with a long chain.

There are two distinct types of phospholipids currently used: synthetic one and natural phospholipid.

Natural Phospholipids

- **PS –** Phosphatidyl serine
- **PC-** phosphatidyl choline (lecithin)

Synthetic phospholipid

- **DOPC-** Dioleoyl phosphatidylcholine
- **DSPC-** Di stearoyl phosphatidyl choline
- **DLPC-** Di lauryl phosphatidyl choline
- **DOPE-**Dioleoyl phosphatidyl ethanolamine

2. Cholesterol

Cholesterol is the most important part for the formation of cell membrane. It provides the fluidity, rigidity and stability to biological membrane.

CLASSIFICATION OF LIPOSOMES [13,14]

A) On the basis of size, lamellae

Table 1- Based on Size & Lamellarity

Based on Size & Lamellarity	**Size**
Multi lamellar large vesicles (MLV)	More than 0.5 µm
Oligo lamellar vesicles (OLV)	0.1-1.0 µm
Uni lamellar vesicles (UV)	All size ranges
Small Uni lamellar vesicles (SUV)	20-100 nm
Large Uni lamellar vesicles (LUV)	More than 100nm
Giant Uni lamellar vesicles (GUV)	More than 1.0 µm

Multi Vesicular vesicles (MVV)	More than 1.0 µm

B) Based upon method of liposome preparation.

Table 2- Method of preparation of liposomes

S. no	Types of vesicles	Method of Preparation
1	REV	Reverse phase evaporation
2	MLV-REV	MLV from reverse phase evaporation
3	VET	Preparation of vesicle by extrusion technique
4	FPV	Vesicles made by French pressure
5	FUV	Prepared from fusion
6	DRV	Dehydration–rehydration method

C) Depending upon application and its composition

i) **Conventional: -**Neutral/negative charged phospholipid.

ii) **Fusogenic:** - with envelopes of sendai virus.

iii) **Cationic:** - cationic lipids with DOPE

METHOD OF PREPRATION [15,16]

Several methods are used to design the liposomes. Some steps are involved for the preparation of liposomes.

- Lipids drying occur from the organic liquid as solvent.
- Lipid's dispersion into the aqueous Media.
- Purification takes place to prepared liposomes.
- Final product analysis takes place.

Other methods are also involved in liposomes preparation.

- Passive loading
- Active loading

Below are explanations of a couple of them:

Mechanical dispersion method: - By this method, generally in organic solvent liposomes were dissolved and separated by film disposition. Upon evaporation of solvent, the mixture of solid-lipid hydrated to aqueous phase.

- **Lipid hydration method**: -Most commonly way for MLV preparation. In this, organic solvent was used such as chloroform or ethanol in which liposomes were to be dissolved and then solution of lipid become dried for preparation of a thin film with a temp. Of 45- 60º Celsius around the bottom of RBF. For the complete removal of solvent, nitrogen gas is generally used. When the film becomes hydrated with the adding of any specific buffer and the solution rotate at high RPM. Hydration takes place when the temp. may be 60- 70◦Celsius.
- **Micro- emulsification:** - This is used to prepare the SLV. In this method, the composition of lipid by using high stressed produced by homogenizer and the equipment used for this purpose known as micro- fluidizer.In this, two solutions were generally proposed and these are- PR liposomes. For the formation of suspension of liposomes, coating the lipid to carrier in which it is soluble and change the material into free- flowing state. For the removal of water at low temp. &press. Freeze- drying (lyophilization) is generally used and this method is applicable for thermo labile substances.
- **Sonication:** - In this method, particles present in the solution will break with the help of sound waves or energy. By using the sound waves, the particles present in the solution are agitated and electrical signal converts into physical vibration. This method helps to mix the solution properly, and increase the rate of dissolution of solid

particles into the liquid.

Generally, two techniques are used: -

a- Probe sonication: - Used to sonicate the suspension which needs high energy.

b- Bath sonication: - This method is used for lipid having large volume.

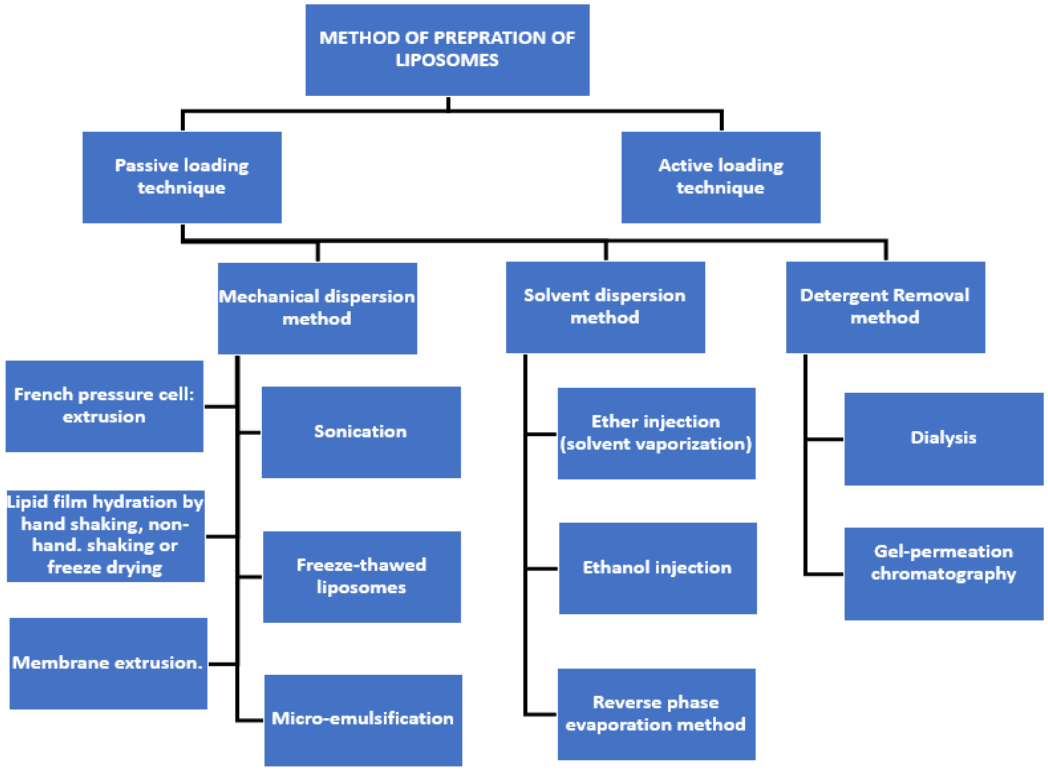
- **French pressure cell:** - In this method, extrusion of multi-lamellar vesicles through the small orifice. In this, observation of protein does not take place during sonication due to the already involvement in sonication.

Solvent dispersion method-

- **Ethanol injection**: -Essential for the manufacture of SUV's. Ethanol Solution of lipid that is to be injected to the buffer through the needle and then the ethanol solution was dispersed in phospholipid and water.
- **Ether injection:** - Making single-layer vesicles is a great usage for this kind of preparation. This involves slowly injecting an immiscible organic solution into an aq. phase by using the narrow needle having temp. In which organic solvent were vaporised.

Detergent removal method: - For the solubilisation of lipids, the detergent at critical micelle concentration(CMC) were used. After removal of detergent, LUVs are prepared formulation and the detergent removal take place by the method of dialysis. This process leads to the uniform preparations of liposomes.

Figure 2. Preparation method of liposomes.



Applications[16]

The major aim to produce the liposome is -enhance stability, pharmacokinetic and distribution of drug in systemic circulation.

Various applications of liposome are given.

- **Targeted at specific site.**

Liposomes play a major role for drug delivery at a specific site by reducing normal tissue damage to obtain the effective and safe therapy.

- **Sustained release drug delivery**

For sustained release of drug, liposomes can be formulated to produce the long-term effect and get concentrated in plasma

at a therapeutic level to get optimum therapeutic effect.

- **Cancer therapy: -**

In cancer drugs in general, liposomes are important; the cytotoxic medicine can distribute throughout the body and also affects the normal cells which can have toxic effect and various side effects occurs. The liposomes of cytotoxic drug can increase the life of symposiums also protect the medicinal metabolism.

- **Pulmonary application: -**

Liposomes are very useful for distributing the drug in lungs.

- **Food and farming industry: -**

Liposome plays a major role in food & farming industry to develop new taste and modify the food colour and odour and controlled release of flavour. Liposome used to entrap the component which is unstable such as anti-oxidants, flavours, anti-microbial.

MARKATED FORMULATION OF LIPOSOMES

Table 3: Market product of liposome.

S. no.	BRANDS	DRUGS
1.	Amphotec, AmbisomeTM , AmphocilTM, AbelcetTM	Amphotericin B
2.	DaunoXomeTM,Daunoxome	Daunorubicin citrate
3.	DepoDur	Morphine
4.	Doxil,EvacetTM	Doxorubicin
5.	Estrasorb	Estradiol
6.	Mikasome	Amikacin
7.	NyotranTM	Nystatin
8.	VincaXome	Vincristine

EVALUATION OF LIPOSOME [1,17,18]

To guarantee their consistent in vitro and in vivo performance, the liposomal formulation and processing for a given function are characterised. A liposomal drug product can be evaluated based on a few of the factors listed below:

Table 4: Evaluation parameters of liposome.

Evaluation Parameters	Method
Size & Size distribution	SEM, TEM, AFM, Freeze fracture technique, cryo-transmission electron microscopy
Entrapment efficiency	Dialysis, centrifugation
Vesicle shape and lamellarity	freeze fracture electron microscopy & 31P-Nuclear magnetic resonance analysis
In-vitro release study	Dialysis method, Franz diffusion cell
Evaluation of Zeta potential	Electrophoretic light scattering
Uniformity & Rigidity of Bilayers	NMR, DSC, FT-IR

2. CONCLUSION

It is being revealed that liposomes are incredibly effective medication delivery vehicles. Notwithstanding of their solubility characteristics, the versatility of their behaviour can be utilised to deliver drugs by any route of administration as well as every pharmacological material. Clinical acceptance is now being attained by liposomes that have improved drug delivery to illness sites due to their extended circulation residence durations. Additionally, liposomes help target specific sick cells at the disease site. The delivery of genes and medications using liposomes shows promise and will undoubtedly continue to advance in the future. However, we can conclude that liposomes have undoubtedly confirmed their place among current delivery systems based on the therapeutic uses and goods that are currently on the market.

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