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REVIEW ARTICLE

Liposomes: Emerging Trends in Novel Drug Delivery with Present and Future Challenges

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ABSTRACT

Today, nanotechnology is a commonly used buzz world in numerous fields of science and everyday life, and fairly recently in drug delivery. During the last few decades liposomes have attracted great interest and investigated as ideal models for biological membranes as well as efficient carriers for drugs, diagnostics, vaccines, nutrients and other bioactive agents. Liposome has been under investigation for many years and number of drugs and genes investigated for controlled release with liposomal formulation is very extensive. Liposome has been investigated for use in cancer treatment, have been shown to reduce the side effects and have been researched for the delivery of protein and neuclic acids. Like all other colloidal system, liposomes suffer from various short comings including interaction with lipoproteins, having high critical micelle concentration that limits stability and limit availability of inexpensive pharmaceutical grade lipids. This review will provide an overview of lipid based vesicle system followed by some of more interesting opportunities and application of it.

Key Words: Carrier Systems, Liposomes, Phospholipid, Encapsulation, Applications, Lipid Vesicles, Manufacturing Techniques.

INTRODUCTION

Liposomes have been widely investigated since 1970 as drug carrier for improving the delivery of therapeutical agents to specific site s in the body. Liposomes are most developed nana carriers for novel and targeted drug delivery. As a result, numerous improvements have been made, thus making this technology potentially useful for the treatment of certain disease in clinic. The success of liposome as drug carriers has been reflected in number of liposome based formulations, which are commercially available or are currently undergoing clinical trials. Liposomes have been receiving a lot of interest as a carrier for advanced drug delivery. This is due to several advantageous characteristics of liposomes such as ability to incorporate not only water soluble but also lipid soluble agents, specific targeting to the required site in the body and versatility in terms of fluidity, size, charge and number of lamellae. The name liposome is derived from Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Liposomes are artificially prepared vesicle with an aqueous inner layer surrounded by lipid bilayer membrane. The term described by Dr Alec D Bangham in 1961.

Various amphipathic molecules have been used to form liposome. The drug molecules can either be encapsulated in aqueous space or intercalated into the lipid bilayer.

Structure and Composition of Liposome [2,7,12]

Among the novel drug delivery systems, liposomes seem to have the best potential to accommodate both water and lipid soluble compounds to protect the liposome-encapsulated drug from metabolic degradation and to act as a delivery mechanism, releasing active ingredients slowly and in a controlled manner. There are number of structural and nonstructural components of liposomes and majorly structural components of biological membrane such as phospholipids are used. Phospholipids, the comer stone of the liposome lipid bilayer. Usually extracted from egg yolk or soy bean oil consist of a hydrophilic head portion covalently attached to two hydrocarbon tails representing the lipophilic

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portion. Aggregation in a bilayer structure occurs by orientation of the hydrophilic head groups towards the aqueous environment & lipophilic hydrocarbon chains sequestered inside, described as follow in figure 1. Molecule of phospholipids are not soluble in water and in aqueous media they align themselves closely in planner bilayer sheets in order to minimize the unfavorable action between the bulk aqueous phase and long hydrocarbon fatty chain. Formation of such a configuration provides the vesicle with the lowest potential energy state through solvation of the polar head groups and hydrophobic interactions of the lipid chain.

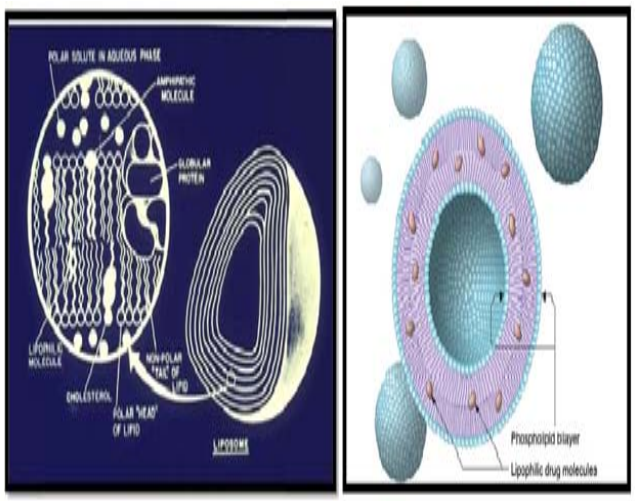

Figure 1: Structure of liposome

(A) Phosphatidylcholine (PC) is the most commonly phospholipids employed in liposomes, and can be obtained from both natural and synthetic sources.

PC is zwitterionic and consists of a hydrophilic head group with a quaternary ammonium moiety choline, which is linked to a glycerol via a phosphoric ester.

The stability of the liposome membrane depends on the packing of the hydrocarbon chains of the lipid molecules. The hydrocarbon chain length and degree of saturation of the acyl chains influences at which temperature, the main transition temperature (Tm), the membrane transforms from a fully extended and closely packed "gel phase" to a liquid crystalline disordered "fluid phase". In general, fluid membranes are more permeable to solutes than rigid bilayers.

Natural phosphatidylcholine extracted from egg yolk or soy bean oil or its semisynthetic derivatives represents the main constituent in various liposomal formulations. The chemical structure of naturally occurring phosphatidylcholine has a glycerol moiety attached to two acyl chains which may be saturated or unsaturated. Each may have between 10 to 24 carbon atoms, together forming the hydrophobic (lipophilic) portion of the molecule. The charged phosphate and choline moieties form the hydrophilic "head".

The fatty acid chains, depending on their length and degree of saturation, can exist in the gel phase in which the lipids are rigid, impermeable and easily aggregated upon storage or in the more fluid liquid crystalline phase.

Cholesterol is frequently added in minute quantities to most liposomal formulations to increase the fluidity of the liposomal gel phase enhance the retention of hydrophilic particles and to stabilize the bilayer membrane in a manner similar to that of biological membranes. [16, 21]

Table 1: The most common glycerophospholipids

The esterified group	Name of the phospholipid	Abbreviation	Net charge at pH 7
-H	Phosphatidicacid	PPA	Negative
-CH2CH2N$^+$(CH3)3	Phosphatidylcholine	PC	Zwitterionic
-CH2CH2NH3$^+$	Phosphatidylethanolamine	PE	Zwitterionic
-CH2CHOHCH2OH	Phosphatidylglycerol	PG	Negative
-HC6H5(OH)5	Phosphatidylinositol	PI	Negative
-CH2CHNH3$^+$COO-	Phosphatidylserine	PS	Negative

The table describes the most common phospholipids with their esterified group and net charge. The charge of the lipid used in liposome formation dictate the surface charge of the liposomes.

The surface charge of liposomes can be tailored by replacing phosphatidylcholine (PC) partly with negatively or positively charged lipids, which induces electrostatic repulsion and stabilization against liposome fusion.

(B) Cholesterol (Chol) and other employed lipids: Cholesterol (Chol) is one of the commonly used lipids in liposome formulations, and its incorporation into the lipid bilayer has a major effect on the liposome properties. The presence of Chol in the lipid bilayer enhances the stability and form highly ordered and rigid membrane with fluid like characteristics.

Chol molecular structure with the four hydrocarbon rings makes it strongly hydrophobic. The presence of the hydroxyl group (OH) attached to the end of Chol makes that part weakly hydrophilic. [3]

Chol does not by itself form bilayer structure, but can be incorporated into the lipid bilayers at

concentrations up to 1:1 molar ratio. Therefore other phospholipids are needed to form a bilayer.
Due to its amphiphatic properties, Chol inserts itself in the bilayer with its OH-group oriented towards the aqueous core, and the rigid hydrophobic tail toward the phospholipid bilayers. [14]

(C) Charge inducers and steric stabilizers: Stearylamine, dicetylphosphate, solulan C-24 and diacylglycerol are commonly used to impart either a negative or a positive surface charge. Since it is a well-known fact that negatively charged and positively charged liposomes are more rapidly uptaken by the reticulo-endothelial system as compared to neutral liposomes, charge inducers are used to overcome this problem. Also they proved to be useful in reducing aggregation as neutral liposomes show higher tendency to undergo aggregation.

(D) Other substances
In case, the drug is very prone to oxidation, antioxidants e.g. tocopherol, butylated hydroxy toluene and stabilizers are used. The use of preservatives is very common to increase the shelf-life of liposomal formulations.

Historical Perspectives [9,11,8,6,18]
The history of liposomes goes back to mid 1960's and the credit goes to Bangham and his coworkers. They were discovered when Bangham and R. W. Horne were testing the institute's new electron microscope by adding negative stain to dry phospholipids in presence of suitable solvents form bilayered membranes which finally curl-on to form unilamellar or multilamellar vesicles. The resemblance to plasma lemma was obvious, and microscopy became first evidence.

The history of liposomes can be divided into three periods:
Genesis, Middle age and Modern era.
Genesis (1968-75)

The physiochemical characterization of liposomes had been carried out in this period. Moreover, thin lipid film hydration method had been developed to prepare multilamellar vesicles. (MLVs).
Middle Age (1975 – 85)

Modification that gives best result that leads increase liposomal utility by basic research that increased the understanding of their stability and interaction characteristic within the system. The time became evidence of discovery of various alternative methods for the preparation of liposomes.

Modern Era (1985 onwards)

Today, liposomes are used successfully in various scientific disciplines, including mathematics and theoretical physics, biophysics, chemistry, colloid science (stability, thermodynamic of finite systems), biochemistry and biology (excretion, cell function, trafficking and signaling, gene delivery and function).

Mechanism of liposome formation [11]

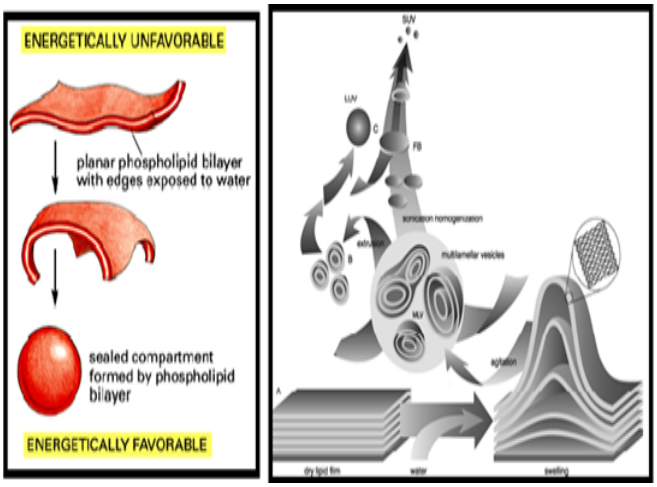

Figure 2: Mechanism of formation of liposome

Advantages of Liposome
1. Liposomes are increased efficacy and therapeutic index of drug (Actinomycin-D).
2. Liposome is increased stability via encapsulation.
3. Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and non immunogenic for systemic and non-systemic administrations.
4. Liposomes are reduction in toxicity of the encapsulated agent (Amphotericin B, Taxol).
5. Liposomes help to reduce exposure of sensitive tissues to toxic drugs.
6. Site avoidance effect.
7. Flexibility to couple with site-specific ligands to achieve active targeting.
8. Provides selective passive targeting to tumor tissue (liposomal doxorubicin).

Disadvantages of Liposome
1. Production cost is high.
2. Leakage and fusion of encapsulated drug / molecules.
3. Sometimes phospholipid undergoes oxidation and hydrolysis like reaction.
4. Short half-life.
5. Low solubility.
6. Fewer stables.

Classification of liposome [18]

Table 2: Classification of liposomes based on size and lamellarity [7]

MLV	Multilamellar large vesicles	(>0.5 µm)
OLV	Oligolamellar vesicles	(0.1–1 µm)
UV	Unilamellar vesicles	(all size range)
SUV	Small unilamellar vesicles	(20–100 nm)
MUV	Medium-sized unilamellar vesicles	-
LUV	Large unilamellar vesicles	(>100 nm)
GUV	Giant unilamellar vesicles	(>1 µm)
MVV	Multivesicular vesicles	(usually >1 µm)

The table describes the classification of liposome based on size and lamellarity, methods of preparation and therapeutical aspect.

Table 3: Classification of liposomes based on method of preparation

REV	Single or Oligolamellar vesicles made by Reverse phase Evaporation method
MLV-REV	Multilamellar large vesicles made by Reverse phase Evaporation method
SPLV	Stable plurilamellar vesicles
FATMLV	Frozon and Thawed MLV
VET	Vesicle prepared by extrusion technique
DRV	Dehydration-Rehydration Method

Method of Liposome Preparation and Drug Loading

The preparation of all types of vesicular systems requires the input of energy.

Generally all the methods of liposome preparation involve three basic stages

1. Drying down of mixture of lipids from an organic solvent.
2. Dispersion of lipids in aqueous media.
3. Separation and purification of resultant liposomes.

The various methods of preparation of liposomes are as under

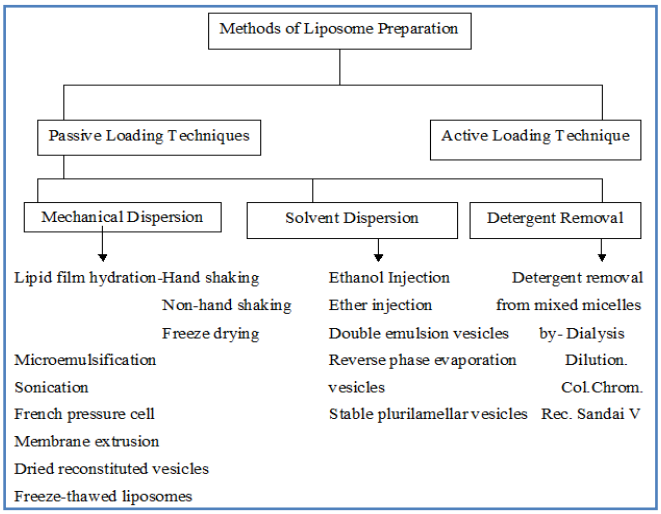

Figure 3: Method of Liposome Preparation

Characterization of Liposomes [18]

Liposome prepared by one of the preceding method must be characterized. The most important parameters of liposome characterization include visual appearance, turbidity, size distribution, lamellarity, concentration, composition, presence of degradation products, and stability.

Table 4: Liposome Characterization

CHARCTERIZATION PARAMETERS		ANALYTICAL METHODS / INSTRUMENTATION
Chemical Characterization		
Concentration	Phospholipid	Barlett/Stewart assay, HPLC
	Cholesterol	Cholesterol oxidase assay,HPLC
	Drug	Method as in individual monograph
Phospholipid	Peroxidation	UVabsorbance, TBA, iodometric, GLC
	Hydrolysis	HPLC,TLC, Fatty Acid Conc.
Cholesterol auto-oxidation		HPLC,TLC,
Ant-oxidant degradation		HPLC,TLC,
pH		pH meter
Osmolarity		Osmometer
Physical Characterization		
Vesicle	Size & Surface morphology	TEM, Freeze fracture electron microscopy
	Size distribution	DLS,Zetasizer,TEM,PCR,gel permeation,exclusion
Surface charge	Free flow electrophoresis	
Electric surface potential &pH	Zeta potential measurement, pH probes	
Lamellarity	SAXS, ^{31}NMR, Freeze fracture EM	
Phase behavior	Freeze fracture EM,DSC	
% Entrapment Efficiency	Minicolumn centrifugation, gel exclusion, ion exchange, protamine aggregation, radiolabelling	
Drug release	Diffusion	
Biological Characterization		
Sterility	Aerobic or anaerobic cultures	
Pyrogenicity	LAL test	
Animal toxicity	Monitoring survival rates, Histopathology	

Challenges [8]

The lipid based drug delivery system is versatile drug carrier with significant potential. only few products have come up with the stage of commercial production.

1) Large scale production

Preparation of liposomes involves various steps like evaporation of solvent system under reduced pressure(vacuum), preparation of thin lipid film, sonication etc.

These steps are difficult to carry out at large scale level especially the preparation of thin film. So, it is difficult to scale up liposome production from laboratory level to large-scale production level. Also the use of organic solvent is a big issue and adding organic solvents such as chloroform, methanol etc to solubilise and mix lipids is not

recommended in such a high concentrations as per the regulatory norms. The solvents not only affect the chemical structure of entrapped substance but it also present in final formulation and produce toxicity but also influence the stability.

2) Stability

Liposomes itself has a advantage for increasing the stability of unstable drugs like tretinoin but the phospholipids used for their production are very prone to oxidation and/or hydrolysis. Therefore lipid based products cannot be stored for a longer period. In some cases, however, the products are available in lyophilized form which has to be reconstituted prior to use. Liposomes not only pose physical instability problems but also show chemical instability.

3) Uptake by Reticulo-endothelial system

For drug delivery, liposomes can be formulated as a suspension, as an aerosol, in a semisolid form such as a cream, gel or a dry powder and these can be administered. After systemic administration, liposomes are typically recognized as foreign particles and consequently endocytosed by cells of the mononuclear phagocyte system (MPS), mostly fixed Kupffer cells in the liver and spleen. For this reason, a search for liposomes that could evade rapid uptake by the MPS started and few lipid compositions that prolonged liposome blood- circulation times have been discovered. By decrease in size in nano foam and PEG-coated or sterically stabilized liposomes are good examples in this regard.

Using judicious selection of components with appropriate physicochemical characteristics, lipid based drug delivery system can be formulated trigger based system. The phase transition temperature is also one of the challenges to formulate novel formulation. Novel engineering approaches involving the incorporation of transition temperature lowering component as well as metallic drug loading schemes to achieve high encapsulation efficiencies are helpful in facillating the implementation of liposomes with enhanced trigger functionally in the clinic.

Application of Liposomes [8]

1. Liposome as drug/protein delivery vehicles.
- Controlled and sustained drug release
- Enhanced drug solubilization
- Altered pharmacokinetics and bio-distribution
- Enzyme replacement therapy and bio-distribution
- 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.
- Immuno-adjuvant
- Immuno-modulator
- Immuno-diagnosis

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.

Marketed formulations

Product	Drug	Company
Ambisome™	Amphotericin B	NeXstar Pharmaceuticals, Inc., CO
Doxil™	Doxorubicin	Sequus Pharmaceuticals, Inc., C.A.
DaunoXome™	Daunorubicin	NeXstar Pharmaceuticals, Inc., CO
Epaxel™	Hepatitis A Vaccine	Swiss Serum Institute, Switzerland
ELA-Max™	Lidocaine	Biozone Labs, CA, USA
MiKasome™	Amikacin	NeXstar Pharmaceuticals, Inc., CO
Abelcet™	Amphotericin B	The Liposome Company, NJ

CONCLUSION

Liposomes are one of the unique drug delivery system, which can be of potential use in controlling and targeting drug delivery. Liposomes are administrated orally, parenterally and topically as well as used in cosmetic and hair technologies, sustained release formulations, diagnostic purpose and as good carriers in gene delivery various drugs with liposomal delivery systems have been approved. Nowadays liposomes are used as versatile carriers for targeted delivery of drug. The development of 'pharmaceutical' liposomes is currently a growth area.

The novel approaches provide also powerful strategy & platform to develop the multifunctional liposomes. Technological advances such as introduction of remote loading methods, pegylated

liposomes and targeted liposomes provided additional advantages. In addition to modulating toxicity, pharmacokinetic and biodistribution liposomal delivery has shown promise as mechanism to overcome multidrug resistance. Furthermore, liposomes are more promising delivery vehicles for novel therapeutic agents such as siRNA and drug that lack of aqueous solubility.

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