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Research Article

Liposomes: Present Prospective and Future Challenges

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Abstract

Liposomes are microscopic (unilamellar or multilamellar) vesicles that are formed as a result of self-assembly of phospholipids in an aqueous media resulting in closed bilayered structures which are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. Liposomes have been considered as one of the most outstanding, versatile and flexible carrier systems, which offer wide opportunity for the delivery of multifarious molecules and applications. The present review focuses upon preparation and characterization of liposomes plus challenges associated with liposomal delivery

Key words: Vesicular drug Carriers, Phospholipids, Active targeting.

BACKGROUND

The rising number of complications associated with drugs from varied chemical and biological background not only made scientists worldwide to search for newer molecules but also to discover the new ways and means for the proper delivery of molecules. With the help of new delivery systems known as novel drug delivery systems (NDDS) both old and new molecules can be delivered to the site in demand in a defined manner. With this targeted delivery, the molecules can be made to produce the desired effect without disturbing the delicate bio-environment. The investment on drug delivery research in contrast to search for the basic therapeutic molecules of synthetic origin may well prove to be less taxing in terms of money, labor and time. Moreover,

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hitherto “molecules as magic bullets“, conceived by Nobel laureate Paul Ehrlich, way back in 1905, has only been remained a hypothesis.

The various drug delivery objectives such as selectivity, specificity, target-ability and safety improved by way of monitoring the distribution, controlling the release and modifying the interaction of therapeutic molecules with receptor have been approached through different angles and dimensions. This led to the development of an array of approaches based on varied physio-chemical and biological tools and techniques which can be used to deliver the drug to the desired target site with almost no or reduced toxicity. Among many available colloidal drug delivery systems, a class based on phospholipids has fetched much more attention than other systems because of their many meritorious features. These vesicular systems have displayed their potential to a great extent in delivering the various drugs to the target site. Liposomes, niosomes, aquasomes, transfersomes, ethosomes are some to mention.

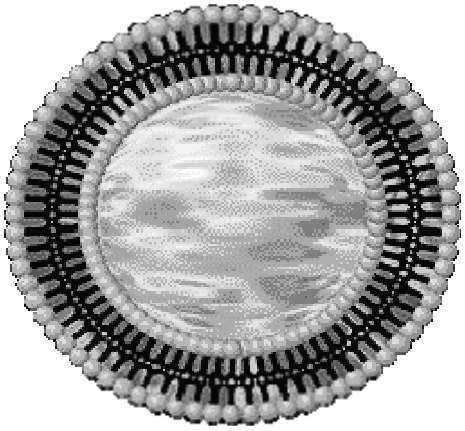


Figure 1: Structure of liposome

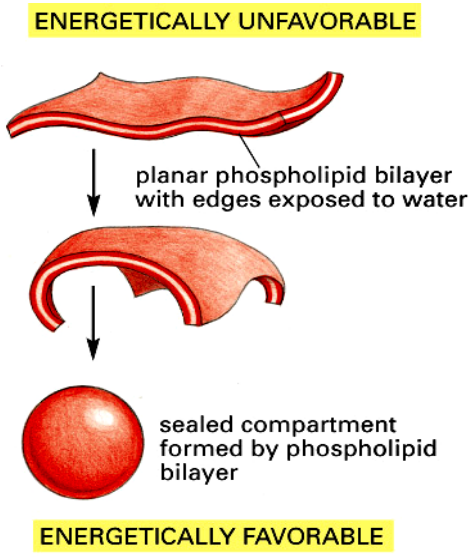


Figure 2: Mechanism of formation of liposome

The liposomes have emerged as most practically useful carriers for in-vivo drug delivery as majority of reports has concentrated on the use of phospholipid vesicles or liposomes as potential drug carrier systems [1]. Liposomes or lipid based vesicles (Figure1) are microscopic (unilamellar or multilamellar) vesicles that are formed as a result of self-assembly of phospholipids in an aqueous media resulting in closed bilayered structures [2, 3]. The assembly into closed bilayered structures is a spontaneous process [4] and usually needs some input of energy in the form of physical agitation, sonication, heat etc. Since lipid bilayered membrane encloses an aqueous core, both water and lipid soluble drugs can be successfully entrapped into the liposomes. The lipid

soluble or lipophilic drugs get entrapped within the bilayered membrane whereas water soluble or hydrophilic drugs get entrapped in the central aqueous core of the vesicles [5].

Historical Perspectives

The history of liposomes goes back to mid 1960's and the credit of their birth goes to Bangham and his coworkers, who discovered that phospholipids in presence of suitable solvents form bilayered membranes which finally curl-on to form unilamellar or multilamellar vesicles. 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). [6,7]. Liposomes were widely used to study the nature of biological membrane because of close resemblance of bilayered membrane with the biological membrane.

Middle Age (1975 – 85)

Liposome's utility was improved following basic research that increased the understanding of their stability and interaction characteristic within the system [8]. This period also dealt with the discovery of various alternative methods for the preparation of liposomes. Also, due to the availability of vast knowledge about the physio-chemical properties of liposomes, their behavior within the body, their interaction with the cells, attempts had been made to improve their performance as drug carrier systems [8,9,10].

Table 1 Various Marketed Formulations of Liposomes

Product	Drug	Company
Ambisome™	Amphotericin B	NeXstar Pharmaceuticals, Inc., CO
Abelcet™	Amphotericin B	The Liposome Company, NJ
Amphocil™	Amphotericin B	Sequus Pharmaceuticals, Inc., C.A.
Doxil™	Doxorubicin	Sequus Pharmaceuticals, Inc., C.A.
DaunoXome™	Daunorubicin	NeXstar Pharmaceuticals, Inc., CO
MiKasome™	Amikacin	NeXstar Pharmaceuticals, Inc., CO
DC99™	Doxorubicin	Liposome Co., NJ, USA
Epaxel™	Hepatitis A Vaccine	Swiss Serum Institute, Switzerland
ELA-Max™	Lidocaine	Biozone Labs, CA, USA

Modern Era (1985 onwards)

Today, liposomes are used successfully in various scientific disciplines, including mathematics and theoretical physics (topology of two-dimensional surfaces floating in a three dimensional continuum), biophysics (properties of cell membranes and channels), chemistry (catalysis, energy conversion, photosynthesis), colloid science (stability, thermodynamic of finite systems), biochemistry (function of membrane proteins) and biology (excretion, cell function, trafficking and signaling, gene delivery and function). Ambisome^{TM}, a parenteral amphotericin-B based liposomal product was first in the race, followed by number of other products which are either at the stage of clinical trials or are already in the market (Table1). Moreover, renaissance in the liposome research is promising many more products to come in the near future [8].

Mechanism of Vesicle Formation

It has been proved that phospholipids spontaneously form closed structures when hydrated in aqueous media. Because phospholipids are amphipathic (both hydrophilic and hydrophobic) in nature, their thermodynamic phase properties and self-assembling characteristics evoke entropically driven sequestration of hydrophobic regions into spherical bilayers [11]. In other words, unfavorable interactions come into play between lipid molecules and water molecules. The self-assembling action of phospholipid molecules into bilayered sheets leads to lowering of unfavorable interaction between the solvent and long hydrocarbon fatty chains thus acquiring a state of lower energy and almost maximum stability. Well known amphiphiles include soaps, detergents and polar lipids (lecithins, kephalins) [8]. Further, to gain a completely stable state, bilayer sheets start folding or curl-on itself to form closed sealed bilayered vesicles enclosing a central aqueous core as depicted in figure 2 [12,13].

This phenomenon can be understood in quantitative terms by considering the critical micelle concentration (CMC) of phosphatidylcholine in water. The CMC is defined as the concentration of the lipid in water (usually expressed as moles per liter) above which the lipid forms either micelles or bilayer structures rather than remaining in solution as monomers. The CMC of dipalmitoylphosphatidylcholine has been measured by Smith and Tenford and found to be $4.6x10^{-10}$ M in water. This value is in agreement with those obtained for similar amphiphiles. Clearly, this is a very small number indicating the overwhelming preference of this molecule for a hydrophobic environment such as that found in the core of a micelle or bilayer.

Design and Development of Liposomes

The ultimate identity of any liposomal system and hence its properties are determined by the various factors. All these variables, directly or indirectly, have their effect on the formation of

liposomes. Therefore, it is necessary that these variables must be carefully controlled during the design of liposomes. Some of these factors are shown below.

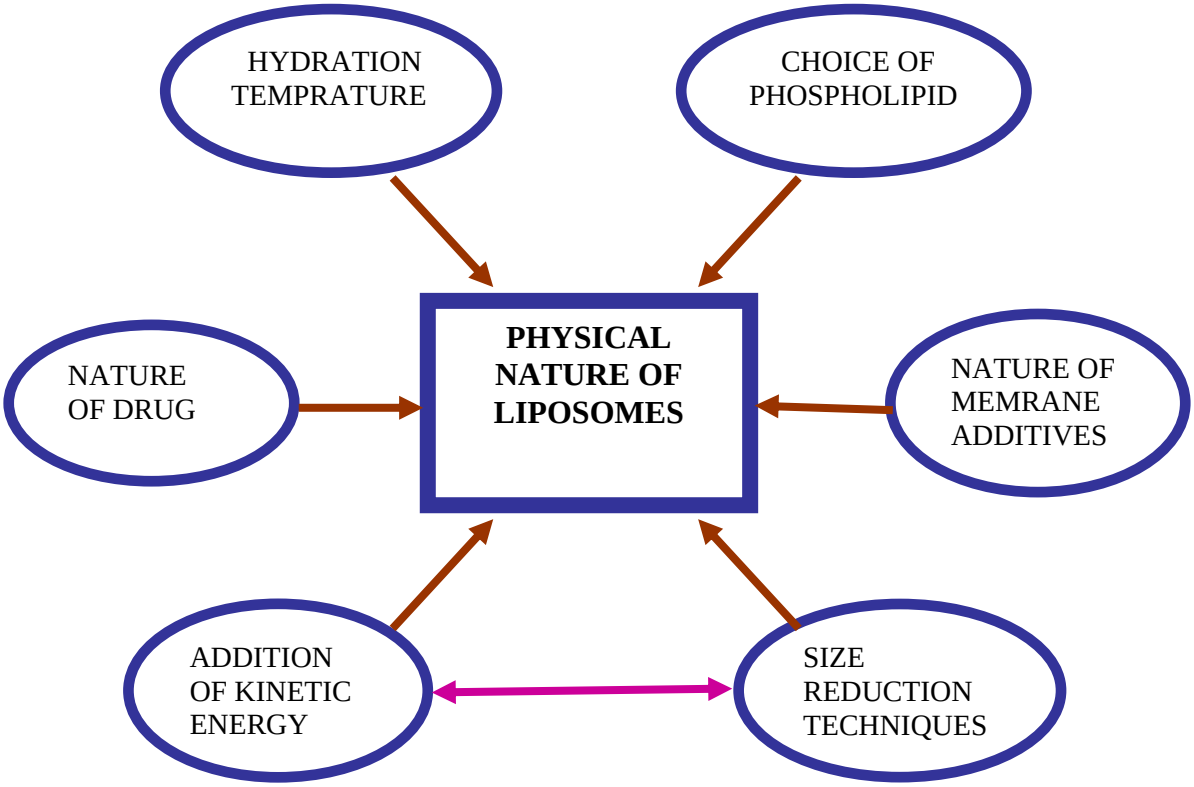


Figure 3: Factors effecting the formation of liposomes

In other words, during the formulation of liposomes all these variables must be optimized in order to obtain the best possible formulation with maximum stability and entrapment efficiency. The design of drug delivery system should always be taken from the past biology of the system. For example, the anticancer drugs are targeted using liposomes to the specific vascular structure of tumor tissue. Another example includes the use of liposomes to target the drug to liver and spleen in leishmaniasis, as particulate uptake by liver and spleen is a known fact. Once a correlation is obtained between the liposomal surface and the resulting biological response, more specific forms of targeting that involve the incorporation of molecular recognition elements may be undertaken. Stealth liposomes having a coating of polyethylene glycol have been widely exploited for the tumor targeting as they have low uptake by reticulo-endothelial system and spleen [14]. In the similar manner, the pharmacodynamics and pharmacokinetics of liposomes can be changed by inclusion of charge inducers and steric stabilizers like dicetylphosphate (DCP), stearylamine (SA), solulan C-24 etc.

Materials

a) Membrane forming components

Phospholipids: bilayer formers

Phospholipids that are the major components of the biological membranes are the building blocks of the liposomes. The phospholipids have tubular shape owning to the presence of two acyl chains attached to a polar head and on hydration, results into a bilayered membrane. Two types of phospholipids are there i.e. phosphodiglycerides and sphingolipids along with their corresponding hydrolysis products [15].

Classification of phospholipids

(a) Neutral phospholipids e.g. Sphingomyelin, Phosphatidylethanolamine and Phosphatidylcholine.

(b) Negatively charged phospholipids e.g. Dipalmitoyl phosphatidylcholine, Dipalmitoyl phosphatidyl acid (DDPA), Distearoyl phosphatidyl choline (DSPC), Dioleoyl phosphatidyl choline (DOPC) etc.

(c) Positively charged phospholipids e.g. 1,2-dihexadecyl-N,N-dimethyl–N-trimethyl amine methyl ethanol amine etc.

b) Membrane Additives (Sterols)

Cholesterol is the most commonly used sterol, which is included in the liposomal membranes. It has been called as the ‘motar’ of bilayers because by virtue of its molecular shape and solubility properties, it fills in empty spaces among the phospholipid molecules, anchoring them more strongly into the structure [16]. Cholesterol is an amphipathic molecule and inserts itself into the membrane with its hydroxyl groups oriented towards the aqueous phase and aliphatic chain aligned parallel to acyl chains of the phospholipid molecules. [17]. In other words, cholesterol increases the transition temperature of the system by making the membrane more ordered [18]. Cholesterol reduces this type of interaction to a great extent and provides both physical and biological stability.

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.

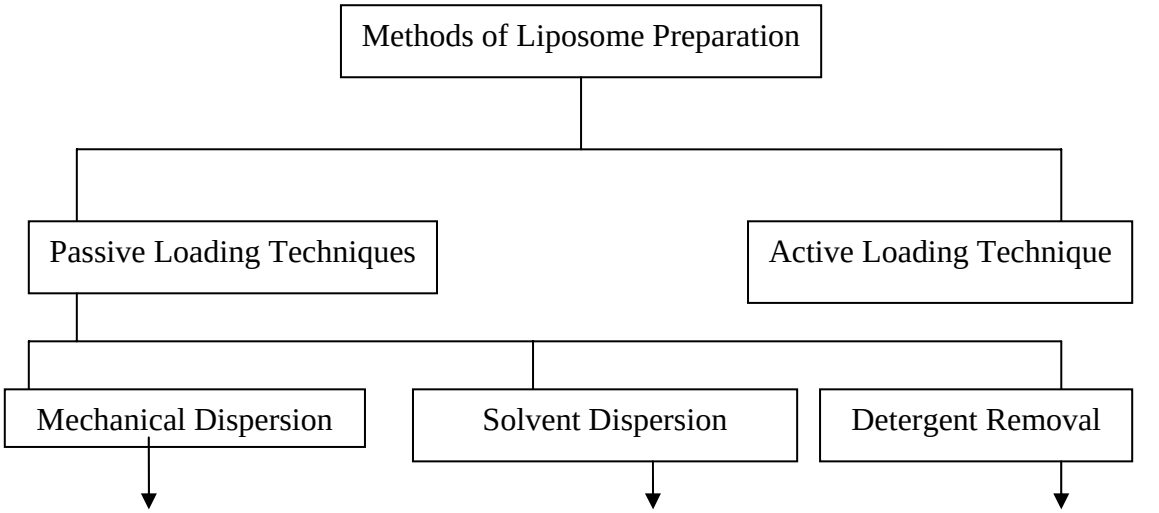
(d) Other substances

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. [19].

Preparation of liposomes

The preparation of all types of vesicular systems requires the input of energy [4]. 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. [20].The various methods of preparation of liposomes are as under [21]



Characterization of Liposomes

The behavior of the liposomes, both in physical and biological system, to a great extent depends upon various factors such as size, shape, lamellarity, entrapment volume etc (Table1). Therefore,

liposomes are characterized for these parameters to determine their in-vivo behavior to certain extent [21].

Table 2 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	UV absorbance,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 exachange,protamine aggregation,radiolabelling
Drug release		Diffusion
Biological Characterization		
Sterility		Aerobic or anaerobic cultures
Pyrogenicity		LAL test
Animal toxicity		Monitoring survival rates, Histopathology

Liposomal Delivery: Future Challenges

Although liposomes have proved their potential as drug delivery vehicles, only few products have come up with the stage of commercial production. Some to mention are Daunoxome, Ambisome, Doxil, Epaxel, etc [8]. There are basically three major problems that we come across with the liposomal delivery systems i.e. uptake by reticulo-endothelial system, large-scale production and instability of phospholipids that pose as a hurdle in their commercial development.

1) 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, which seems to be the most promising route for these carrier systems, 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. This fate is very useful for delivering drugs to these cells but, in general, excludes other applications, including site-specific drug delivery by using ligands expressed on the liposome surface in order to bind to receptors over-expressed on the diseased cells. 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. PEG-coated or sterically stabilized liposomes are good examples in this regard.

2) Large scale production

Preparation of liposomes involves various steps like evaporation of solvent system under reduced pressure, 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 adding organic solvents such as chloroform, methanol etc to solubilize and mix lipids is not recommended in such a high concentrations as per the regulatory norms.

3) Stability

Liposomes itself has a advantage for increasing the stability of unstable drugs like tretinoin but the phosphdolipids 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. Moreover, it has been discovered that electrostatic stabilization of liposomes cannot provide adequate stability to liposomes in the presence of disintegration substances such as the proteins and enzymes encountered in in-vivo applications [8].

CONCLUSION

In summary, this article reviewed the possible applications of liposomes and discussed, in brief, some problems associated with formulation and development. An encouraging sign is the increasing number of clinical trials involving liposome and lipid-based products. With the newer developments in the field, several companies are actively engaged in expansion and evaluation of liposome products for use in anticancer and antifungal therapy and for prophylaxis (vaccines) against diseases. Further refinements in the liposome technology will spur the full-fledged evolvement of liposomes as drug carriers.

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