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Review Article

An Overall Review on Liposomal Drug Delivery System

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ABSTRACT

Liposomes have been widely investigated since 1970 as drug carriers for improving the delivery of therapeutic agents to specific sites in the body. There are numerous improvements for the treatment of certain diseases by using this technology. Liposomes can be defined as simple microscopic vesicles in which lipid bilayer structures are present with an aqueous volume entirely enclosed by a membrane, composed of lipid molecule. Liposomes are biodegradable and essentially non-toxic vehicles. They can encapsulate both hydrophilic and hydrophobic materials, and are utilized as drug carriers in drug delivery. Liposomes are generally classified based upon structure, method of preparation, composition and application. The prepared liposomes are characterized for visual appearance, liposomal size distribution, lamillarity, liposome stability, entrapped volume and surface charges. The liposomes have many applications which increase its importance over other formulations. The current pharmaceutical preparations of liposome-based therapeutic systems mainly result from our understanding of lipid-drug interactions and liposome disposition mechanisms. This review mainly focuses on the advantages and limitations of liposomes, different methods for the preparation and applications of liposomes. In this review discussion was made about the stability problems of liposomes.

INTRODUCTION

The name liposome is derived from two Greek words: 'Lipos' meaning fat and 'Soma' meaning body. Liposomes were first described by British hematologist Dr Alec D Bangham in 1961.

Liposomes are defined as "simple microscopic vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecule." 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. A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell [1, 2].

Advantages of liposomes [3]

1. Provides selective passive targeting to tumour tissue (liposomal doxorubicin).
2. Liposome increases efficacy and therapeutic index of drug (Actinomycin-D).
3. Liposome increased stability via encapsulation.
4. Liposomes are biocompatible, completely biodegradable, non-toxic, flexible and non immunogenic for systemic and non-systemic administrations.
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.

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 stability

Reasons to use Liposome as Drug Carriers

Solubilisation

Liposomes may solubilise lipophilic drugs that would otherwise be difficult to administer intravenously.

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Protection: Liposome-encapsulated drugs are inaccessible to metabolising enzymes; conversely, body components (such as erythrocytes or tissues at the injection site) are not directly exposed to the full dose of the drug.

Duration of action: Liposomes can prolong drug action by slowly releasing the drug in the body. Directing potential Targeting options change the distribution of the drug throughout the body.

Internalisation: Liposomes are endocytosed or phagocytosed by cells, opening up opportunities to use 'liposome dependent drugs'. Lipid based structures (not necessarily liposomes) are also able to bring plasmid material into the cell through the same mechanism (non-viral transfection systems).

Amplification: Liposomes can be used as adjuvant in vaccine formulations

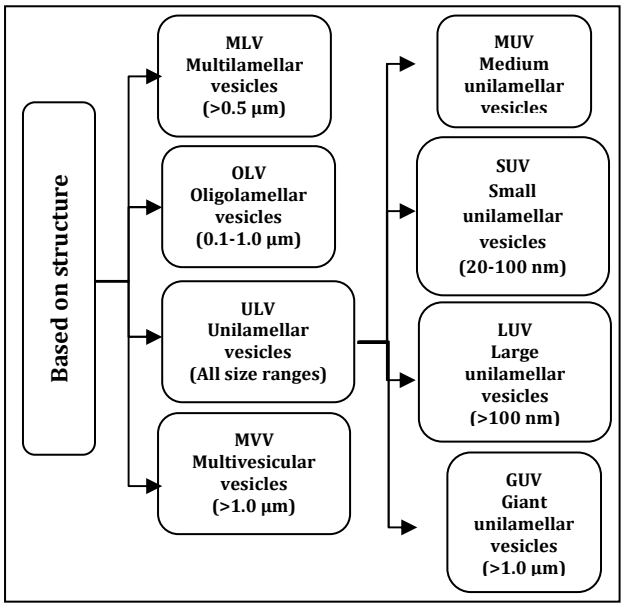

Figure 1: Classification of liposomes based on structural parameters.

Classification of Liposomes

Liposomes can be classified either on the basis of their structural properties or on the basis of the preparation method used. These two classification system are in principle, independent of each other. Depending on the selection of lipids, the preparation technique, and preparation conditions, liposomes can vary widely in size, number, and position of lamellae. These parameters influence the behavior of liposomes both in vivo and in vitro.

Liposome classification based on structural features

MLV (Multi lamellar large vesicles)
OLV (Oligo lamellar vesicles)
ULV (Uni lamellar vesicles)
SUV (Small uni lamellar vesicles)
MUV (Medium sized uni lamellar vesicles)
LUV (Large uni lamellar vesicles)
GUV (Giant uni lamellar vesicles)
MVV (Multi vesicular vesicles)

Liposome classification based on method of liposome preparation

REV Single or oligolamellar vescile made by reverse phase evaporation method.
MLV / REV Multilamellar vesicles made by reverse phase evaporation method.
SPLV Stable plurilamellar vesicles.
FATMLV Frozen and thawed MLV
VET Vesicles prepared by extrusion method.
FUV Vesicles prepared by fusion
FPV Vesicles prepared by french press
DRV Dehydration- rehydration vesicles
BSV Bubblesomes

Classification of liposome based on composition and application

Depending upon composition and applications they have been classified into following types

1. Conventional liposomes
2. Fusogenic liposomes
3. pHsensitive liposome
4. Cationic liposome
5. Long circulating liposome
6. Immuno liposome

Mechanism of Liposome Formation [16]

Liposomes are vesicular structures consisting of hydrated bilayers. Liposomes structures used for pharmaceutical purposes consist of a phospholipid backbone. But other classes of molecules can form bilayer based vesicular structures as well. On the other hand not all the hydrated phospholipids form bilayer structures. Other forms of self aggregation such as inverted hexagonal phases or micelles with completely different properties can occur. The common feature that all bilayer forming compounds share is their amphilicity. They have defined polar and nonpolar regions. In water the hydrophobic regions tend to self aggregate and the polar-regions tend to be in contact with the water phase. Israelachvili and coworkers defined critical packing parameter p by

$$P = v/ a0lc$$

Where v is the molecular volume of the hydrophobic part, a0 is the optimum surface area per molecule at the hydrocarbon water interface, and lc is the critical half thickness for the

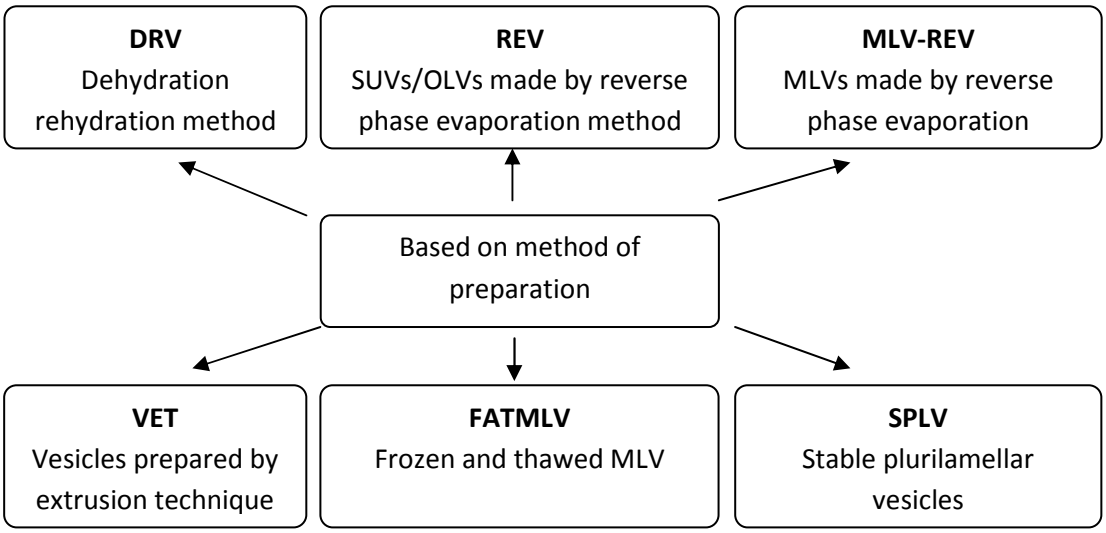

Figure 2: Classification of liposomes based on method of preparation

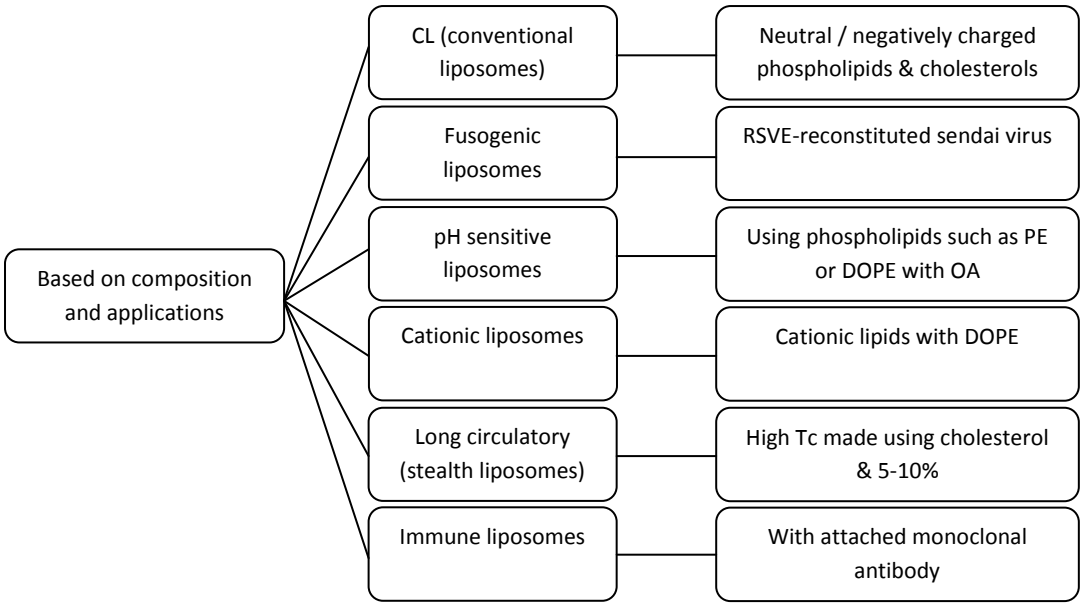

Figure3: Classification of liposomes based on composition and applications.

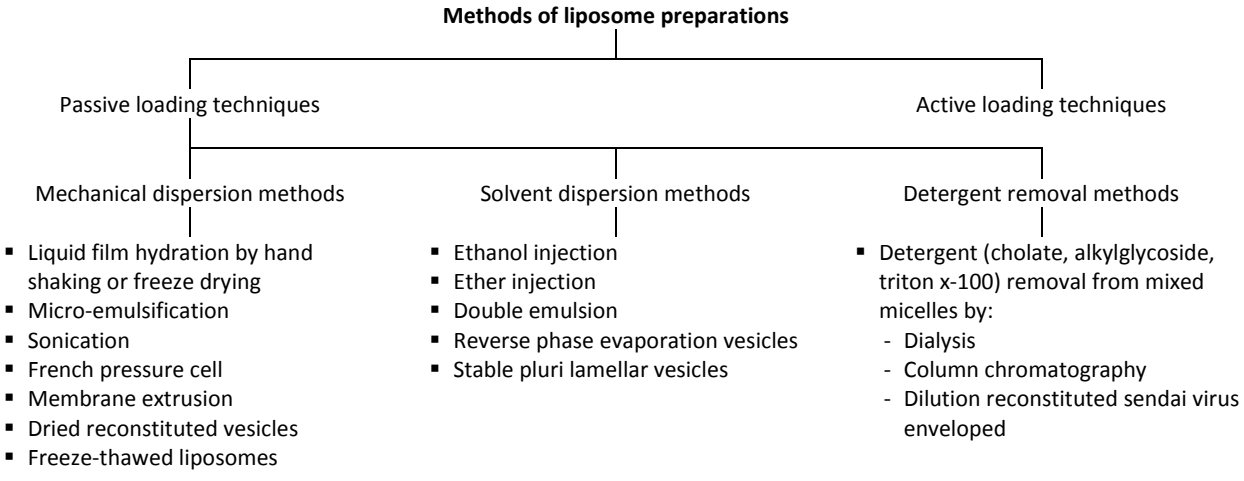

Figure 4: Different methods of liposome preparation

hydrocarbon region which must be less than the maximum length of the extended lipid chains. For $p < 1/3$, spherical micelles are formed. In this category fall single chain lipids with large head group areas eg; lysophosphatidylcholine. For $1/3 < ½$ globular or cylindrical micelles are formed. Double chain "fluid state" lipids with large head area ($1/2 < p < 1$) form bilayers and vesicles. This occurs also with double chain "gel state" lipids with small head groups and $p \sim 1$. For $p > 1$ inverted structures such as the inverted hexagonal phase can be observed. An additional condition required for bilayer formation is that the compound can be classified as a no soluble swelling ampiphile.

Raw Materials for Formation of Liposomes

Generally, liposome composition includes natural and/or synthetic phospholipids (Phosphatidylserine, Phosphatidylinositol).

Phosphatidylcholine (also known as lecithin) and phosphatidylethanolamine constitute the two major structural components of most biological membranes. Liposome bilayers may also contain other constituents such as cholesterol, hydrophilic polymer, conjugated lipids and water. Cholesterol has been largely used to improve the bilayer characteristics of the liposomes. It improves the membrane fluidity, bilayer stability and reduces the permeability of water soluble molecules through the membrane. A clear advantage of liposomes is the fact that the lipid membrane is made from physiological lipids which decreases the danger of acute and chronic toxicity.

Methods of liposomes preparations:

The correct choice of liposome preparation method depends on the following parameters:

1) The physico chemical characteristics of the material to be entrapped and those of the liposomal ingredients;
2) The nature of the medium in which the lipid vesicles are dispersed;
3) The effective concentration of the entrapped substance and its potential toxicity;
4) Additional processes involved during application/ delivery of the vesicles;
5) Optimum size, polydispersity and shelf-life of the vesicles for the intended application
6) Batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

Classical Technique

There are four classical methods of liposome manufacture. The difference between the various methods is the way in which lipids are drying down from organic solvents and then redispersed in aqueous media. These steps are performed individually or are mostly combined.

Hydration of a Thin Lipid Film: Bangham [4, 5]

This is the original method which was initially used for liposomes production. A mixture of phospholipid and cholesterol were dispersed in organic solvent. Then, the organic solvent was removed by means of evaporation (using a Rotary Evaporator at reduced pressure). Finally, the dry lipidic film deposited on the flask wall was hydrated by adding an aqueous buffer solution under agitation at temperature above the lipid transition temperature. This method is widespread and easy to handle, however, dispersed phospholipids in aqueous buffer yields a population of multilamellar liposomes (MLVs) heterogeneous both in size and shape (1–5 _m diameter). Thus, liposome size reduction techniques, such as sonication for SUVs formation or extrusion through polycarbonate filters forming LUVs were useful to produce smaller and more uniformly sized population of vesicles.

Reverse-Phase Evaporation (REV) Technique [6, 7]

A lipidic film is prepared by evaporating organic solvent under reduced pressure. The system is purged with nitrogen and the lipids are re-dissolved in a second organic phase which is usually constituted by diethyl ether and/or isopropyl ether. Large unilamellar and oligolamellar vesicles are formed when an aqueous buffer is introduced into this mixture. The organic solvent is subsequently removed and the system is maintained under continuous nitrogen. These vesicles have aqueous volume to lipid ratios that are 30 times higher than sonicated preparations and 4 times higher than multilamellar vesicles. Most importantly, a substantial fraction of the aqueous phase (up to 62% at low salt concentrations) is entrapped within the vesicles, encapsulating even large macromolecular assemblies with high efficiency.

Solvent (Ether or Ethanol) Injection Technique

The solvent injection methods involve the dissolution of the lipid into an organic phase (ethanol or ether), followed by the injection of

the lipid solution into aqueous media, forming liposomes. The ethanol injection method was first described in1973. The main relevance of the ethanol injection method resides in the observation that a narrow distribution of small liposomes (under 100 nm) can be obtained by simply injecting an ethanolic lipid solution in water, in one step, without extrusion or sonication. The ether injection method differs from the ethanol injection method since the ether is immiscible with the aqueous phase, which is also heated so that the solvent is removed from the liposomal product. The method involves injection of ether-lipid solutions into warmed aqueous phases above the boiling point of the ether. The ether vaporizes upon contacting the aqueous phase, and the dispersed lipid forms primarily unilamellar liposomes. An advantage of the ether injection method compared to the ethanol injection method is the removal of the solvent from the product, enabling the process to be run for extended periods forming a concentrated liposomal product with high entrapment efficiencies.

Detergent Dialysis

Liposomes, in the size range of 40–180 nm, are formed when lipids are solubilized with detergent, yielding defined mixed micelles. As the detergent is subsequently removed by controlled dialysis, phospholipids form homogeneous unilamellar vesicles with usefully large encapsulated volume. Other methods have been already used for liposomes preparation such as: calcium induced fusion, Nanoprecipitation and emulsion techniques. However, these classical techniques require large amounts of organic solvent, which are harmful both to the environment and to human health, requiring complete removal of residual organic solvent. Furthermore, conventional methods consist of many steps for size homogenization and consume a large amount of energy which is unsuitable for the mass production of liposomes.

Limitations

However, these classical techniques require large amounts of organic solvent, which are harmful both to the environment and to human health, requiring complete removal of residual organic solvent. Furthermore, conventional methods consist of many steps for size homogenization and consume a large amount of energy which is unsuitable for the mass production of liposomes.

Since industrial scale production of liposomes has become reality, the range of liposome preparation methods has been extended by a number of techniques such as Heating Method, Spray drying, Freeze Drying, Super Critical Reverse Phase Evaporation (SCRPE), and several modified ethanol injection techniques which are increasingly attractive.

Heating Method

A new method for fast production of liposomes without the use of any hazardous chemical or process has been described. This method involves the hydration of liposome components in an aqueous medium followed by the heating of these components, in the presence of glycerol (3% v/v), up to 120^0c. Glycerol is a water-soluble and physiologically acceptable chemical with the ability to increase the stability of lipid vesicles and does not need to be removed from the final liposomal product. Temperature and mechanical stirring provide adequate energy for the formation of stable liposomes. Reza Mozafari et al. confirmed by TLC that no degradation of the used lipids occurred at the above mentioned temperatures. The particle size can be controlled by the phospholipid nature and charge, the speed of the stirring and the shape of the reaction vessel. Otherwise, employment of heat abolishes the need to carry out any further sterilisation procedure reducing the time and cost of liposome production [7].

Spray-Drying

Since spray-drying is a very simple and industrially applicable method, the direct spray-drying of a mixture of lipid and drug was applied in the preparation of liposomes. The spray-drying process is considered to be a fast single-step procedure applied in the nanoparticles formulation. Hence, liposomes were prepared by suspending lecithin and mannitol in chloroform. The mixture was sonicated for 8 min (bath sonicator) and subjected to spray-drying on a Buchi 190 M Mini Spray Dryer. The spray-drying conditions were as follows: inlet and outlet temperatures were 120 ^{0}C and 80 ^{0}C, respectively; airflow rate was 700 NI/hr; and the flow rate was 1000 ml/hr. The dried product was hydrated with different volumes of phosphate buffered saline (PBS; pH 7.4) by stirring for 45 min. The main factor influencing the liposomal size was the volume of aqueous medium used for hydration of the spray-dried product. However, mannitol plays an important role in increasing the surface area of the lipid mixture, enabling successful hydration of the spray-dried product, appropriate ratios to form a clear isotropic

monophase solution. Then the monophase solution was sterilized by filtration and filled into freeze-drying vials. In recent study, a laboratory freeze drier was used and freeze-drying process was as follows: freezing at −40 ^{0}C for 8 h; primary drying at −40 ^{0}C for 48 h and secondary drying at 25 ^{0}C for 10 h. The chamber pressure was maintained at 20 Pascal during the drying process. On addition of water, the lyophilized product spontaneously forms homogenous liposome preparation. After investigation of the various parameters associated with this method it is found that the lipid/carrier ratio is the key factor affecting the size and the polydispersity of the liposome preparation. Therefore, TBA/water cosolvent system was used for economy concerns [8].

Modified Ethanol Injection Method

Novel approaches based on the principle of the ethanol injection technique such as the microfluidic channel method, the crossflow-injection technique and the membrane contactor method were recently reported for liposome production.

The Crossflow Injection Technique

The concept of continuous crossflow injection is a promising approach as a novel scalable liposome preparation technique for pharmaceutical application. Wagner et al. used a cross flow injection module made of two tubes welded together forming a cross. At the connecting point, the modules were adapted with an injection hole. The influencing parameters such as the lipid concentration, the diameter of injection hole, the injection pressure, the buffer flow rate, and system performance were investigated. A minimum of buffer flow rate is required to affect batch homogeneity and strongly influencing parameters are lipid concentration in combination with increasing injection pressures, after exceeding the upper pressure limit of the *Modified Ethanol Injection Method.* Novel approaches based on the principle of the ethanol injection technique such as the microfluidic channel method, the crossflow-injection technique, and the membrane contactor method were recently reported for liposome production [9].

Microfluidization

By using a microfluidic hydrodynamic focusing (MHF) platform, Jahn et al. generated liposomes by injecting the lipid phase and the water phase into a microchannel. Microfluidic flow is generally laminar due to the small channel dimensions and relatively low flow rates. Well-defined mixing is then obtained by interfacial diffusion when multiple flow streams are injected in a microchannel. The size of the liposomes was mainly controlled by changing the flow rate.

Stability of liposomes [10-12]

The stability of liposomes in gastrointestinal tract is very important if they are to be used as drug carrier by the oral route. Liposomes should be stable against enzymes found in the GIT, bile salts and gastric acidity. The pancreatic lipase was capable of degrading naturally occurring phospholipids. It has been found that liposomes containing short chain fatty acids were more stable against destructive action of lipase.

Remedies [13, 14]

- Liposomes are used as carriers for drugs and diagnostic agents. In general in early stages of development freshly prepared liposome's are used, however from a pharmaceutical point of view it is important to demonstrate that liposome's can be stored for a long period of time. Few precautions are necessary for the satisfactory production of liposomes for drug entrapment.
- To avoid air oxidation of the fatty acyl groups of the component phospholipids, which produces lyso-compounds and free fatty acids which will modify the liposome structure. The usual precaution is to keep stored lipids as well as prepared liposomes in an atmosphere of nitrogen or inert gas such as argon.
- Oxidation of phospholipids is probably not a major problem, since it can be minimized by preventive and protective measures such as the use of antioxidants.
- Instability of liposomes in colloidal system is due to aggregation and fusion at the molecular level. The simplest way to overcome it is to introduce charge into the lipid mixture. Electrostatic repulsions sufficient to stabilize liposome in vitro. As zeta potential is a very good index of the magnitude of the repulsive interaction between colloidal particles. It is commonly used to assess the stability of colloidal solution
- PH- sensitive liposomal formulations were stabilized by human plasma, probably caused by insertion of apolipoprotein A1 in the

membrane or by exchange of unsaturated lipids with saturated analogues might improve the blood stability of pH- sensitive liposomes.

Applications of liposomes [15,16]

- Liposomes are used as a model, tool, or reagent in the basic studies of cell interactions, recognition processes, and of the mode of action of certain substances
- Improved solubility of lipophilic and amphiphilic drugs. This is possible due to precipitation of the drug or gel formation inside the liposome with appropriate substances encapsulated.
- Passive targeting to the cells of the immune system, especially cells of the mononuclear phagocytic system (in older literature reticuloendothelial system). Examples are antimonials, Amphotericin B, porphyrins and also vaccines, immunomodulators or (immuno) suppressor.
- Sustained release system of systemically or locally administered liposomes [17,18].
  Site-avoidance mechanism: liposomes do not dispose in certain organs, such as heart, kidneys, brain, and nervous system and this reduces cardio-, nephro-, and neurotoxicity.
- Site specific targeting: in certain cases liposomes with surface attached ligands can bind to target cells ('key and lock' mechanism).
- Natural toxins induce strong macrophage response which results in macrophage activation. This can be duplicated and improved by the use of liposomes because small molecules with immunogenic properties (haptens) cannot induce immune response without being attached to a larger particle. Activation of macrophages was proven useful in the treatment of viral, bacterial, and fungal infections as well. Activating factors such as cytokines and lymphokines, including interferon are attached [19,20].
- Small liposomes composed of lipids with long and saturated hydrocarbon chains in mixtures with cholesterol were shown to accumulate at the sites of inflammations. Such liposomes were used for diagnostic purposes
- Long circulation times significantly, i.e. 200-fold, increased the area under curve of drug concentration vs. time and accumulation in various tumours was proportional to the liposome circulation time [21].

Table 1: Marketed Formulations

Various Marketed Formulations of Liposome Product	Drug	Company
Ambisome	Amphotercin B	NeXstar Pharmaceuticals, Inc., CO
Abelcet	Amphotercin B	The Liposome Company, NJ
Amphocil ™	Amphotercin 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

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