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A SHORT REVIEW ON "LIPOSOMES"

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ABSTRACT
Liposomes, sphere – shaped vesicles consisting of one or more phospholipid bilayers, approved for clinical use in 1995. The term Liposome means lipid body. It has been derived on the basis of subcellular particles, Ribosomes. Their size ranges from 25 to 500nm. Liposomes present as an attractive delivery system due to their flexible physiochemical delivery considerations. In this review article, we discussed about Liposome, these are one among the various drug delivery system used to target the drug to particular tissue. The various other drug delivery includes noisome, microparticles, released erythrocytes, pharmacosomes etc. Research on liposome technology has progressed from conventional vesicles to "Second -generation liposomes", in which long circulating liposomes are obtained by modulating the lipid composition, size and charge of the vesicle. This paper summarizes exclusively components, methods of preparation, Drug delivery etc.

KEYWORDS: Drug delivery system using liposomes, advantages and disadvantages of liposomes, applications, Mechanism, Method of preparation of liposomes.

INTRODUCTION

In 1906 Paul Ehrlich introduced the era of development for targeted delivery mechanism that would target drug directly to diseased cell, what he called as a magic bullet.[1-4] Liposomes were first made synthetically in England in 1961 by Alec D. Bangham.[5] "Liposomes are colloidal, vesicular structures composed of one or more lipid bilayers surrounding an equal number of aqueous compartment."[6-7]

Drug is entrapped within the liposome and is released from the liposome for absorption at the intestinal membrane surface. The main component of liposomes are phospholipids which are amphiphilic molecules containing water soluble, hydrophilic head section and a lipid-soluble, hydrophobic tail section. This property of phospholipids give liposomes unique applications in different fields including food, cosmetic, agriculture and pharmaceutics.[8] A significant advantage of liposome is that it can incorporate and release two materials with different solubilities simultaneously. One example for which is the incorporation of two antioxidant agents namely alpha-tocopherol (a lipid – soluble molecule) and glutathione (a water – soluble molecule) in the same lipid vesicle.[8] The properties of liposomes are influenced by various factors, including lipid composition, surface charge, size, and the method of preparation.[9] Liposomes can be formed from naturally-derived phospholipids with mixed lipid chains (like egg phosphatidylethanolamine).[10]

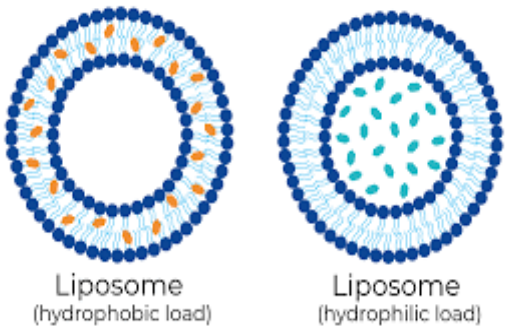

Fig:1

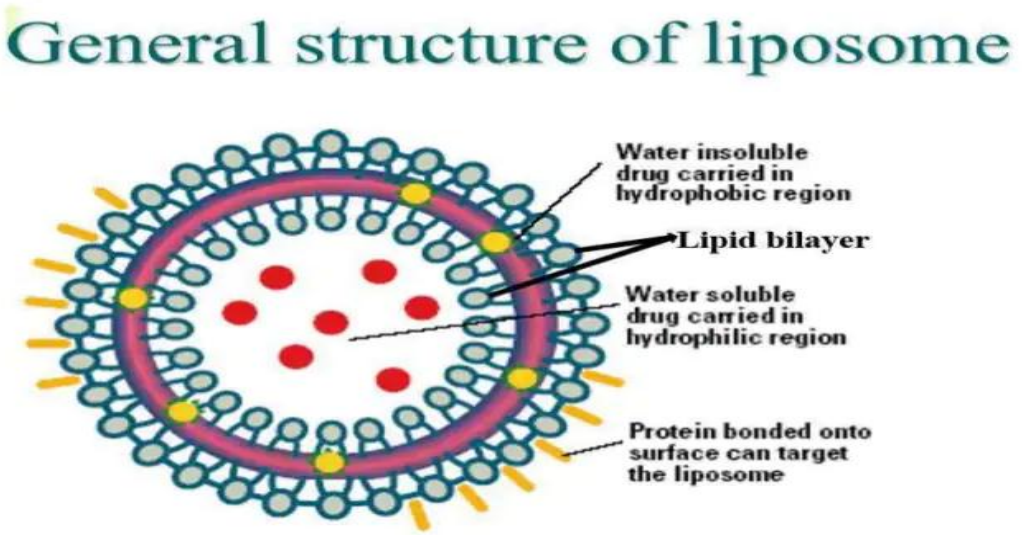

Fig:2

CLASSIFICATION OF LIPOSOME

- **Based on Structural parameters**

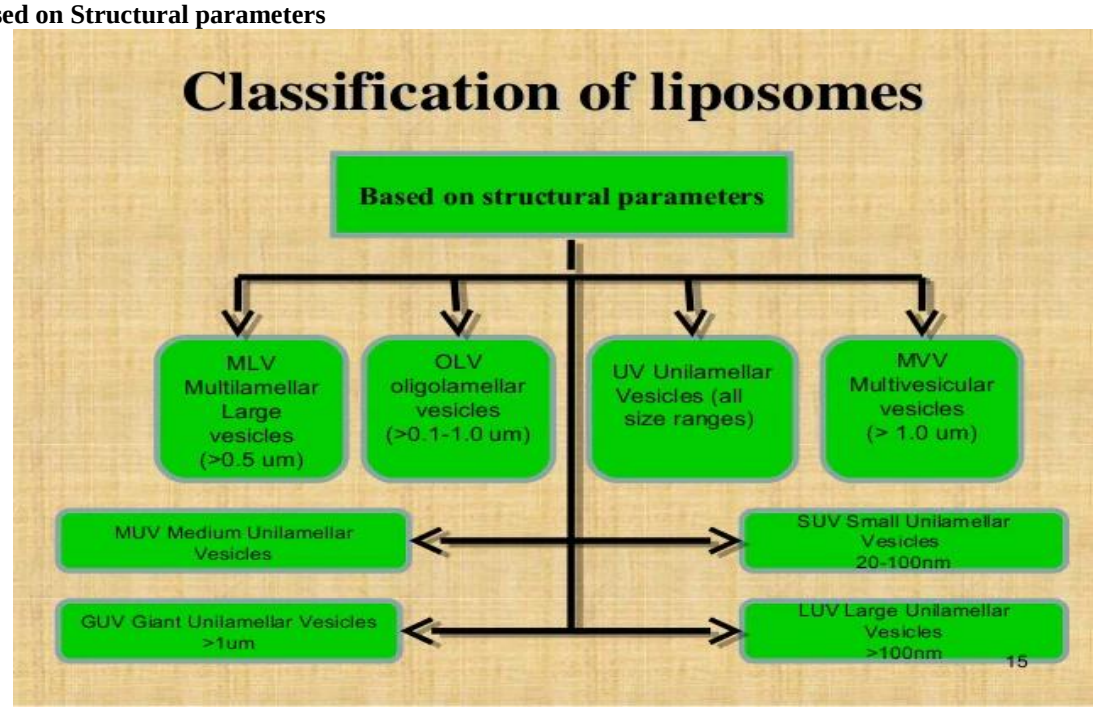

Fig:3

- **Based on method of preparation**

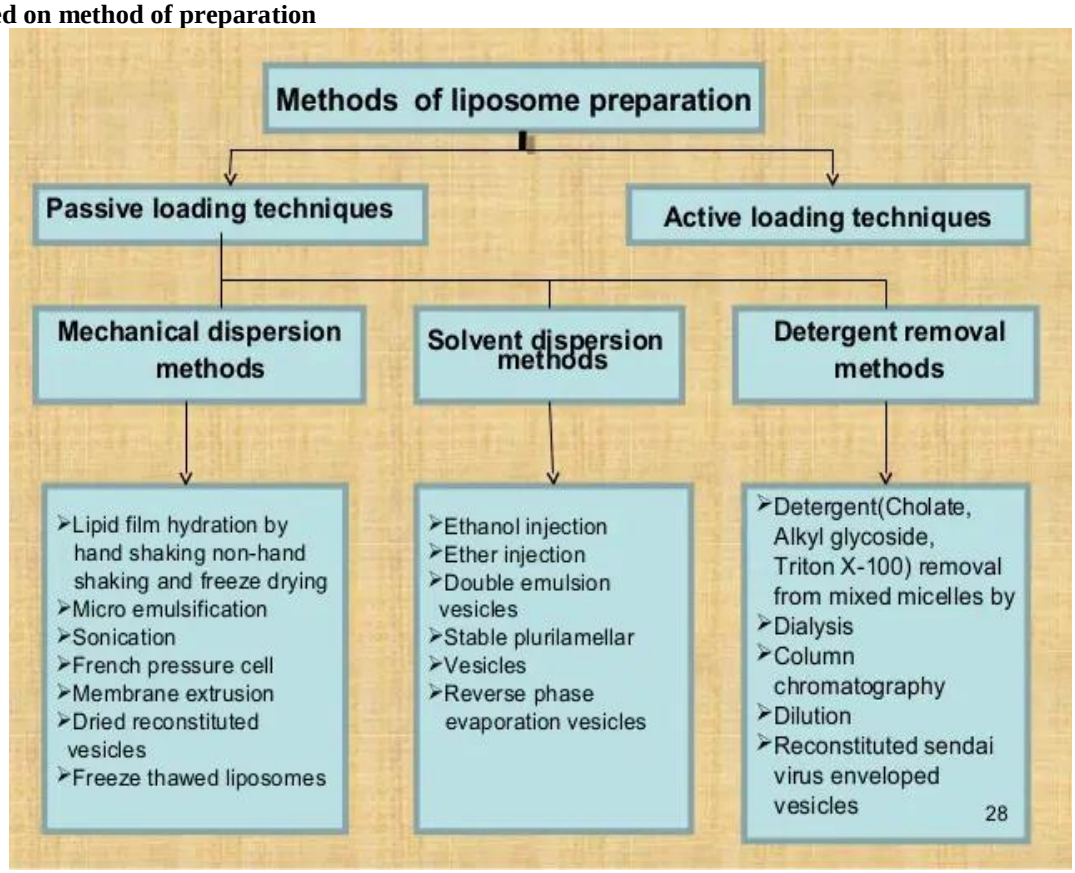

Fig:4

- **Based upon method of composition and application**

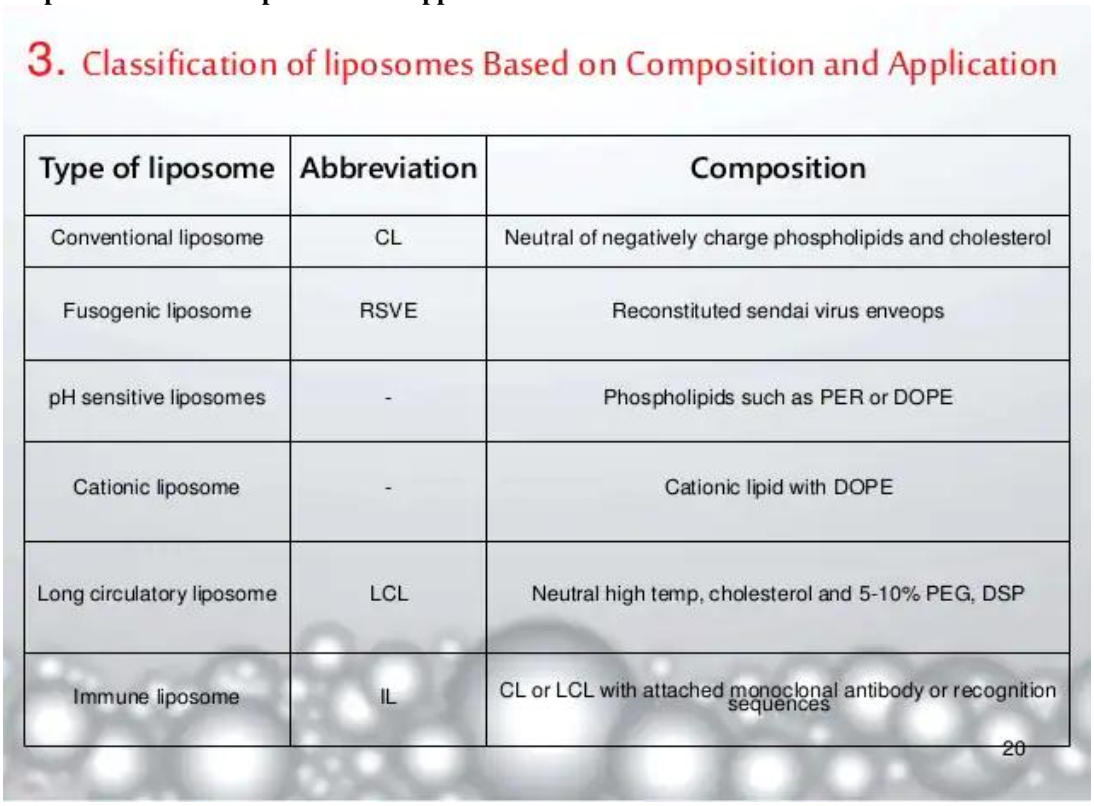

Fig:5

Advantages and disadvantages of liposomes

Table 1 Advantages and disadvantages of liposome [19]

Advantages of liposome	Disadvantages of liposome
Liposomes increased efficacy and therapeutic index of drug (actinomycin-D)	Low solubility
Liposome increased stability via encapsulation	Short half-life
Liposomes are non-toxic, flexible, biocompatible, completely biodegradable, and non-immunogenic for systemic and non-systemic administrations	Sometimes phospholipid undergoes oxidation and hydrolysis-like reaction
Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol)	Leakage and fusion of encapsulated drug/ molecules
Liposomes help reduce the exposure of sensitive tissues to toxic drugs	Production cost is high
Site avoidance effect	Fewer stables
Flexibility to couple with site-specific ligands to achieve active targeting	

Applications of liposomes in medicine and Pharmacology

Applications of liposomes in medicine and pharmacology can be divided into diagnostic and therapeutic applications of liposomes containing various markers or drugs, and their use as a tool, a model, or reagent in the basic studies of cell interactions, recognition processes, and mode of action of certain substances.[25]

Unfortunately, many drugs have a very narrow therapeutic window, meaning that the therapeutic concentration is not much lower than the toxic one. In several cases, the toxicity can be reduced or the efficacy can be enhanced by the use of a suitable drug carrier which alters the temporal and spatial delivery of the drug, i.e., its biodistribution and pharmacokinetics. It is clear from many pre-clinical and clinical studies that drugs, for instance antitumor drugs, parceled in liposome demonstration reduced toxicities, while retentive enhanced efficacy.

Advances in liposome are leading to new applications for the delivery of new biotechnology products, for example antisense oligonucleotides, cloned genes, and recombinant proteins. A vast literature define the viability of formulating wide range of conservative drugs in liposomes, frequently resultant in improved therapeutic activity and/or reduced toxicity compared with the free drug. As a whole, changed pharmacokinetics for liposomal drugs can lead to improve drug bioavailability to particular target cell that live in the circulation, or more prominently, to extravascular disease sites, for example, tumors. Recent improvements include liposomal formulations of all-trans-retinoic acid[26,27] and daunorubicin[28-31], which has received Food and Drug Administration consent as a first-line treatment of AIDS-related advanced Kaposi`s sarcoma. Distinguished examples are vincristine, doxorubicin, and amphotericin B.[32]

PREPARATION OF LIPOSOMES[11-23]

- GENRAL METHOD OF PREPARATION
- SPECIFIC METHODS OF PREPARATION

A) GENERAL METHOD OF PREPARATION

The lipid is dissolved in organic solvent. The solvent is evaporated leaving a small film of lipids on the wall of the container. An aqueous solution of drug is added. In first procedure the mixture is agitated to produce multi lamellar vesicle and then sonicated to get SUVs. In the second procedure the mixture is sonicated and the solvent is evaporated to get LUVs. After extrusion SUVs are formed. Drug can be incorporated into the aqueous solution or buffer if it is water soluble or included in organic solvent if it is hydrophobic. Free drug and liposomes can be separated by gel chromatography.

B) SPECIFIC METHODS

These are classified as 3 types based on the modes of dispersion. They are

1. Physical Dispersion methods
2. Solvent Dispersion methods
3. Detergent Solubilization methods

1) PHYSICAL DISPERSION METHODS: In these methods the aqueous volumes enclosed within lipid membranes is about 5- 10%, which is very small proportion of total volume used for preparation. So large amount of water- soluble drug is wasted during preparation. But lipid soluble drug can be encapsulated to high percentage. In these methods, MLVs are formed and further treatment is required for preparation of Unilamellar vesicles.

Hand Shaken Method: This is the simplest and widely used method. The lipid mixture and charged components are dissolved in chloroform and methanol mixture (2:1 ratio) and then this mixture is introduced in to a 250 ml round bottom flask. The flask is attached to rotary evaporator connected with vacuum pump and rotated at 60 rpm. The organic solvents are evaporated at about 30 degrees. A dry residue is formed at the walls of the flask and rotation is continued for 15 minutes after dry residue appeared. The evaporator is detached from vacuum pump and nitrogen is introduced into it. The flask is then removed from evaporator and fixed onto lypholizer to remove residual solvent. Then the flask is again flushed with nitrogen and 5 ml of phosphate buffer is added. The flask is attached to evaporator again and rotated at about

60 rpm speed for 30 minutes or until all lipid has been removed from the wall of the flask. A milky white suspension is formed finally. The suspension is allowed to stand for 2 hours in order to complete swelling process to give MLVs.

Non-Shaking Method: This is similar to shaking method except that care is taken in swelling procedure. The solution of lipid in chloroform and methanol mixture is spread over the flat bottom of the conical flask. The solution is evaporated at room temperature by flow of nitrogen through the flask without disturbing the solution. After drying water saturated nitrogen is passed through the flask until the opacity of the dried film disappears. After hydration, lipid is swelled by addition of bulk liquid. The flask is inclined to one side, 10 to 20 ml of 0.2M sucrose in distilled water is introduced down the side of the flask and then flask is slowly returned to upright position. The solution is allowed to run gently over the lipid layer on the bottom of the flask. The flask is flushed with nitrogen sealed and allowed to stand for 2 hours at 37 degrees for swelling. After that the vesicles are mixed to yield a milky suspension. The suspension is centrifuged at 1200 rpm for 10 minutes. The layer of MLVs floating on the surface is removed. From the remaining fluid, LUVs are produced.

Freeze Drying: Another method of dispersing the lipid in a finally divided form prior to addition of aqueous media is to freeze dry the lipid dissolved in a suitable organic solvent. The solvent usually used is tertiary butanol. All the above methods produce MLVs. These are too large or too heterogeneous. In order to modify the size, the prepared MLVs are further processed using the following procedures.

PROCESSING OF LIPIDS HYDRATED BY PHYSICAL MEANS

Micro-emulsification of liposomes: An equipment called micro fluidizer is used to prepare small vesicles from concentrated lipid suspension. The lipids can be introduced in to the fluidizer as a suspension of large MLVs. This equipment pumps the fluid at very high pressure through 5micrometer, screen. Then it is forced long micro channels, which direct two streams of fluids collide together at right angles at very high velocity. The fluid collected can be recycled through the pump and interaction chamber until vesicles of spherical dimensions are obtain Sonication: This method reduces the size of the vesicles and imparts energy to lipid suspension. This can be achieved by exposing the MLV to ultrasonic irradiation. There are two methods of sonication.

A) using bath sonicator

B) using probe sonicator.

The probe sonicator is used for suspensions which require high energy in small volume. (eg: high concentration of lipids or viscous aqueous phase) The bath sonicator is used for large volume of dilute lipids. The disadvantage of probe sonicator is contamination of preparation with metal from tip of probe. By this method small unilamellar vesicles are formed and they are purified by ultra-centrifugation.

Membrane Extrusion Liposome: In this method the size is reduced by passing them through a membrane filter of defined pore size. There are two types of membrane filter. The tortuous path type and the nucleation track type. The former is used for sterile filtration. In this random path arise between the criss cross fibers. The average diameter of these fibers is controlled by the density of fibers in the matrix. Liposomes that are larger than the channel diameter get struck when one tries to pass them through such membrane. The nucleation track type is composed of thin continuous sheet of polycarbonate. They will offer less resistance to passage of liposomes as these consist of straight sided pore holes of exact diameter bored from one side to another. This method can be used to process both LUVs and MLVs.

Freeze and Thaw Sonication: This is a method in which rupture and refusing of UVs are done during which the solute equilibrates between the inside and outside. This process increases the entrapment volume and entrapment efficiency. This method will result in the formation of vesicles with in vesicled and vesicle between lamellae. This method can increase the entrapment volume upto 30%.

2) SOLVENT DISPERSION METHODS: In these methods lipids are first dissolved in an organic solution and then brought into contact with aqueous phase containing materials to be entrapped within liposome. At the interface between the organic and the aqueous phases the phospholipids align themselves to form a monolayer, which is important step to form the bilayer of liposome.

Ethanol injection method: This is simple method. In this method an ethanol solution of the lipids is directly injected rapidly to an excess of saline or other aqueous medium through a fine needle. The ethanol is diluted in water and phospholipids molecules are dispersed evenly through the medium. This procedure yields a high proportion of SUVs (about 25nm diameter).

Ether injection: This method is similar to above one. It involves injecting the immiscible organic solution very slowly into an aqueous phase through a narrow needle at temperature of vaporizing of organic solvent. In this method the lipids are carefully treated and there is very less risk of oxidative degradation. The disadvantage is that long time is required for the process and careful control is needed for introduction of lipid solution.

3) DETERGNT SOLUBILIZATION TECHNIQUE: In this method the phospholipids are brought into close contact with the aqueous phase via detergents, which associate with phospholipids molecules. The structures

formed as a result of this association are known as micelles. They are composed of several hundereds of component molecules. The concentration of detergent in water at which micelles start to form is called CMC. Below CMC the detergent molecule exist in free solution. As the detergent molecule is dissolved in water at concentrations higher than the CMC, micelle form in large amounts. As the concentration of detergent added is increased more amount of detergent is incorporated into the bilayer, until a point is reached where conversion from lamellar form to spherical micellar form take place. As detergent concentration is further increased, the micelles are reduced in size.

MECHANISM OF FORMATION OF LIPOSOMES[12-14]

Lipids capable of forming liposomes exhibit a dual chemical nature. Their head groups are hydrophilic and their fatty acyl chains are hydrophobic. It has been estimated that each Zwitter ionic head group of Phosphatidyl choline has on the order of 15 molecules of water weakly bound to it, which explain it's over whelming preference for the water phase. The hydrocarbon fatty acid chains on the other hand vastly prefer each other company to that of H2O. This can be understood by taking the CMC of P.C into account. The CMC of Dipalmitoyl P. found to be 4.6 –10 M in water, which is a small number indicating the over whelming preference of this molecule for a hydrophobic environment such as that found in the core of micelle or bilayer. The free energy of transfer from water to micelle is 15.3 Kcal/mol for Dipalmitoyl PC and13.0Kcal/mol for Dimyristoyl P.C. These results clearly point out the thermodynamic basis for bilayer assembly that has been termed the hydrophobic effect. The large free energy change between a water and a hydrophobic environment explains the over whelming preference of typical lipids to assemble in bilayer structures, including water as much is possible from the hydrophobic core in order to achieve the lowest energy level, hence the highest stability for the aggregate structure.

List of marketed Product of liposomes

Brand name	Active constituent	Manufacturer
Intelectol®	Vinpocetine	Menory Secret Inc., USA
Efudex®	N3-o-toluyl-Fluorouracil	Valeant Pharma. Intl, USA
Amphocil®	Amphotericin B	Sequus Pharmaceuticals, Inc., C.A
Abelcet®	Amphotericin B	Liposome Company NJ,USA
MiKasome®	Amikacin	NeXstar Pharmceuticals, Inc., Co
Ambisome®	Amphotericin B	NeXstar Pharmceuticals, Inc., Co
DaunoXome®	Daunorubicin	NeXstar Pharmceuticals, Inc., Co
ELA-MAX®	Lidocaine	Biozone Labs, CA, USA
Epaxel®	Hepatitis A Vaccine	Swiss SerumInstitute, Switzerland
Lipofen®	Fenofibrate	Kowa Pharma Inc., USA
DC99®	Doxorubicin	Liposome Company NJ, USA
Doxil®	Doxorubicin	Sequus Pharmaceuticals, Inc., C.A
Ambisome®	Amphotericin B	Astellas Pharma Inc., USA

Source: Ravi et al. (2011) and Sharma et al. (2010).

CONCLUSION

Liposomes have been used in a broad range of pharmaceutical applications. Liposomes are showing particular promise as intracellular delivery systems for anti-sense molecules, ribosomes, proteins/peptides, and DNA. Liposomes with enhanced drug delivery to disease locations, by ability of long circulation residence times, are now achieving clinical acceptance. Also, liposomes promote targeting of particular diseased cells within the disease site. Finally, liposomal drugs exhibit reduced toxicities and retain enhanced efficacy compared with free complements. Only time will tell which of the above applications and speculations will prove to be successful. However, based on the pharmaceutical applications and available products, we can say that liposomes have definitely established their position in modern delivery system.

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