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Review Article

# LIPOSOMES: A NOVEL DRUG DELIVERY SYSTEM

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## ABSTRACT

*In this review article we discuss about liposomes, these are one amongst the various drug delivery system used to target the drug to particular tissue .Because structure similarity between lipid bilayer and cell membrane, liposome can penetrate effectively deliver drug to such that a free drug would not penetrate. Liposomes have been widely investigated since1970 as drug carriers for improving the delivery of therapeutic agents to specific sites in the body. Liposomes, which are biodegradable and essentially non-toxic vehicles, can encapsulate both hydrophilic and hydrophobic materials, and are utilized as drug carriers in drug deliverysystems. As a result, numerous improvements have been made, thus making this technology potentially useful for the treatment of certain diseases in the clinics. 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, basic characteristic, marketed formulation and future prospectus and characterization of liposomes plus challenges associated with liposomal delivery. This review would be a help to the researchers working in the area of liposomal drug delivery.*

***KEY WORDS***
*Liposomes, Liposomal drug delivery, Pharmacodynamics, reticulo-endothelial system, Phospholipids, Target Drug Delivery Sytem*

## INTRODUCTION

The method by which a drug is delivered can have a significant effect on its efficacy. Some drugs have an optimum concentration range within which maximum benefit is derived, and concentrations above or below this range can be toxic or produce no therapeutic benefit at all. On the other hand, the very slow progress in the efficacy of the treatment of severe diseases, has suggested a growing need for a multidisciplinary approach to the delivery of therapeutics to targets in tissues. From this, new ideas on controlling the pharmacokinetics, pharmacodynamics, non-specific toxicity, immunogenicity, biorecognition, and efficacy of drugs were generated. These new strategies, often called drug delivery systems (DDS), are based on interdisciplinary approaches that combine polymer science,pharmaceutics, bioconjugate chemistry, and molecular biology. To minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various drug delivery and drug targeting systems are currently under development. Among drug carriers one can name soluble polymers, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, cells, cell ghosts, lipoproteins, liposomes, and micelles.

Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug

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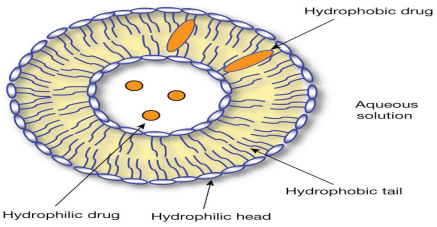
molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids. Participation of nonionic surfactants instead of phospholipids in the bilayer formation results in niosomes. Channel proteins can be incorporated without loss of their activity within the hydrophobic domain of vesicle membranes, acting as a size-selective filter, only allowing passive diffusion of small solutes such as ions, nutrients and antibiotics.

Thus, drugs that are encapsulated in a nanocagefunctionalized with channel proteins are effectively protected from premature degradation by proteolytic enzymes. Liposomes have the distinct advantages of being both nontoxic and biodegradable because they are composed of naturally occurring substances. Biologically active materials encapsulated within liposomes are protected to varying extent from immediate dilution or degradation, suggesting drug carrier systems for the transport of drugs and other bioactive capsules to disease-affected organs. The unique ability of liposomes to entrap drugs both in an aqueous and a lipid phase make such delivery systems attractive for hydrophilic and hydrophobic drugs. Because of advancements in the methods of preparing and formulating liposomes, high-entrapment efficiencies are possible for incorporating drugs into liposomes, creating a tremendous pharmaceutical impact. Furthermore, such encapsulation has been shown to reduce drug toxicity while retaining or improving the therapeutic efficacy.

Several laboratories have reported the use of liposomes as drug carriers in the treatment of cancer, leishmaniasis, metabolic disorders, and fungal diseases. Innovative research in liposomal drugs has led to commercialization of several anticancer therapeutics such as Doxil, Myocet, two liposome-based anticancer drugs; doxorubicin; and an antifungal drug formulation, AmBisome, which is a liposomal formulation of amphotericin B used for systemic therapy. Liposomes may have a use in gene delivery to correct gene-associated disorders or for vaccine therapy.A quantitative entrapment of DNA can be achieved using the preparation of empty liposomes with cationic lipids followed by mixing with DNA or a plasmid of interest. 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 .

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 [1, 2]. The assembly into closed bilayered structures is a spontaneous process 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. Because of its convenience and efficacy, cationic lipid mediated gene delivery technology is a promising system for in vivo gene therapy. Clinical trials of large-size lipid–DNA complexes have mostly shown a lack. 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.




**Fig**

***TYPE OF LIPOSOMES [4]***

Depending upon the structure there are two type of liposomes.

***a) Unilamellar liposomes:***

Unilamellar vesicles has a single phospholipid bilayer sphere
enclosing aqueous solution

***b) Multilamellar Liposomes:***

Multilamellar vesicles have onion structure. Typically, several Unilamellar vesicles will form one inside theother in diminishing size, creating a multilamellar structure of concentric phospholipid spheres separated by layers of water.

***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.

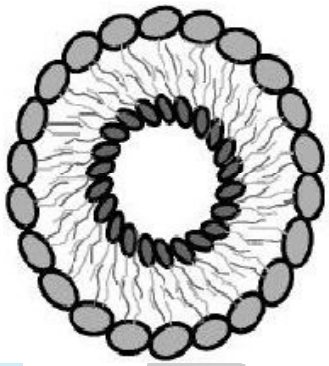


**Fig :- 2. Very Small, Single Layer liposome**

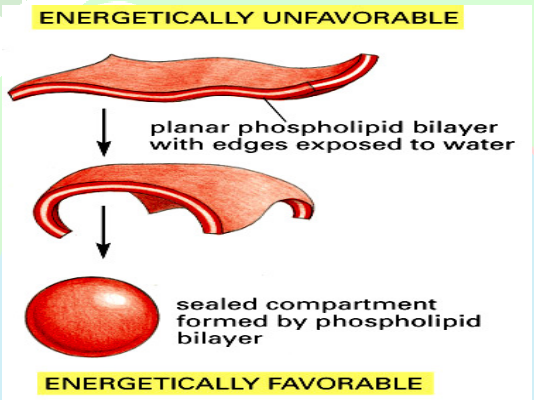
**Table 1: Liposome classification based on structural features**

| | | |
|---|---|---|
| MLV | Multilamellar large vesicles | (>0.5 μm) |
| OLV | Oligolamellar vesicles | (0.1–1 μm) |
| UV | Unilamellar vesicles | (all sizes) |
| 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) |

**Table II: Based on Method of Liposome preparation**

| | |
|---|---|
| REV | Single or Oligolamellar vesicles 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 technique |
| DRV | Dehydration-Rehydration Method |

***Mechanism of Liposome Formation***




Liposomes are vesicular structures consisting of hydrated bilayers.Liposomes structures used for pharmaceutical purposes consist of a phospholipid baskbone. 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 hte 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 hydrocarbon region which must be less tehn the maximum length of the extended lipid chains.

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. 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). 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. 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.

***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.

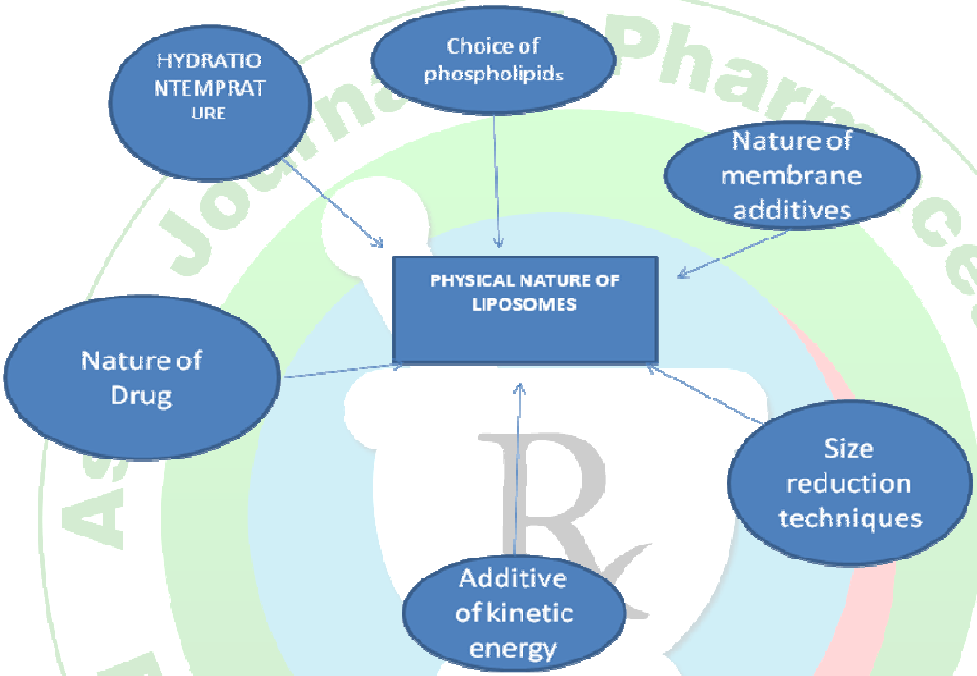



**Figure 3: Factors effecting the formation of liposomes**

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. 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.

***Raw Materials For Formation Of Liposomes***

***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.

***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 . 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. In other words, cholesterol increases the transition temperature of the system by making the membrane more ordered. 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.

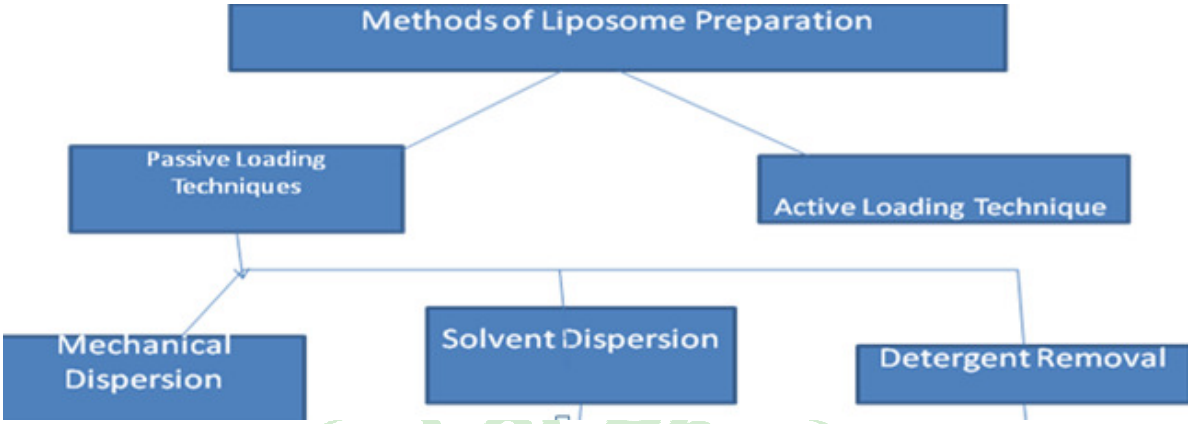
***Techniques of Liposome Preparation(3,4)***

***Preparation of liposomes***

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***




**CHARACTERIZATION OF LIPOSOMES WITH THEIR QUALITY CONTROL ASSAYS**

***A. Biological characterization***

| **Characterization parameters** | **Instrument for analysis** |
|---|---|
| 1. Sterility | Aerobic/anaerobic culture |
| 2. Pyrogenicity | Rabbit fever response |
| 3. Animal toxicity | Monitoring survival rats |

***B. Chemical characterization***

| **Characterization parameter** | **Instrument for analysis** |
|---|---|
| 1. Phospholipids concentration | HPLC/Barrlet assay |
| 2. Cholesterol concentration | HPLC / cholesterol oxide assay |
| 3. Drug concentration | Assay method |
| 4. Phospholipids per oxidation | UV observance |
| 5. Phospholipids hydrolysis | HPLC/ TLC |
| 6. Cholesterol auto-oxidation | HPLC/ TLC |
| 7. Anti-oxidant degradation | HPLC/TLC |
| 8. PH | PH meter |
| 9. Osmolarity | Osmometer |

***C. Physical Characterization***

| **Characterization parameter** | **Instrument for analysis** |
|---|---|
| 1. Vesicle shape, and surface morphology | TEM and SEM |
| 2. Vesicle size and size distribution | Dynamic light scattering ,TEM |
| 3. Surface charge | Free flow electrophoresis |
| 4. Electrical surface potential and surface PH | Zeta potential measurement and PH sensitive probes |
| 5. Lamellarity | P31NMR |
| 6. Phase behavior | DSC, freeze fracture electron microscopy |
| 7. Percent capture | Mini column centrifugation, gel exclusion |
| 8. Drug release | Diffuse cell/ dialysis |

***Stability of Liposomes***

Liposomes face a number of chemical and physical destabilization processes. So liposomes stability is an important consideration while studying liposomes. This aspect of liposomes stability have two aspects physical and chemical stability.

***Physical stability***

Physical processes that effect shelf life include loss of liposome associated drug and changes in size, aggregation and fusion.Aggregation is the formation of larger units of liposome material,these units are still composed of individual liposomes.In principle,this process is reversible ,e.g., by applying mild shear forces, or bychanging the temperature or by binding metal ions that intiaaly induced aggregation. Fusion indicates that new colliodal structure were formed. As fusion is an irreversible process, the original liposomes can never be retrieved.Drug molecules can leak from liposomes. The leakage rate strongly depends on the bilayer composition and the physiochemical nature of the drug. Bilayers in the gel state or those containig substantail (molar ) fractions of cholesterol tend to lose assocaited drug only slowly; liquid state bilayers are more prone to drug loss and are less stable during storage. Bilayer permaebility is not necessarily a constant parameter. Chang in bilayer pernaebility can occur as a result of chemical degredation processes , such as the formation of lypo-PC and FA.

***Chemical stability***

**a) Hydrolyiss of the ester bonds :**

Phosphatidylcholine possesses for ester bons. The two acyl ester bonds are most liable to hydrolysis. The glycerophosphate and phosphocholine ester bonds are more stable. The 1-acyl-lysophosphatidylcholine (LPC) and 2-acyl LPC are both formed at comparable rates.

**b) Lipid peroxidatin of phospholipids:**

The polyunsaturated acyl chains of phospholipids are sensitive to oxidation via free radical reactions. Cyclic peroxides, hydroperoxides, malonilaldehyde, alkanes are the major degradation products. Low oxygen pressure, absence of heavy metals, addition of anti-oxidants, complexing agents (EDTA,etc), quenchers(beta-carotene) of the photo-oxidatio reactions improve resistance against lipid peroxidation.

***Liposomes For Gene Delivery***

It is important to dissect the overall cell uptake process into individual steps. In fact different studies have indicated that successful gene transfer in vitro involves: 1) the packaging of DNA,2) the adhesion of packaged DNA to the cell surface, 3)internalization of DNA, 4) escape of DNA from endosomes if endocytosis is involved, 5) DNA expression in cell nuclei. To perform all of the above steps, liposomes have been explored as a delivery system for DNA as early as in 1979. The encapsulation of plasmid DNA into liposomes and the introduction of poliovirus RNA and SV40 DNA into cells via liposomes were reported between 1979 and 1980.

***Ph Sensitive Liposome Strategy***

Liposomes of various compositions can extensively bind to cell surfaces. For gene transfer, it was established that dioleylphosphatidyl ethanolamine (DOPE) is by far the most efficient lipid for in vitro gene transfection for pH-sensitive liposomes or as lipid helper in cationic liposomes. It has been assumed that the function of phosphatidylethanolamine (PE) is that of a membrane fusion promoter, since in fact this lipid undergoes changes upon acidification. Cholesterol is often essential to achieve sufficient stability of these liposomes. The composition of liposomes may play an important role in their interactions with cells. The size of liposomes and the type of cells are fundamental for an efficient capture by cells. Generally, liposomes are taken up by various endocytosic processes. Professional phagocytes such as macrophages and neutrophils can take up liposomes of various size and charge through active phagocytosis. The vesicular pathway for cellular uptake. After binding to the cell surface, liposomes are internalized into endosomes where they encounter a more acidic pH than in the external medium. Early endosomes generally have an internal pH of 6.50. Their contents are then transferred to a more acidic environment by maturation or vesicular fusion. The last

Cationic Lipid Strategy Liposomes In Cosmetics. endosome environment, with an internal pH of 5.5-6.0, is reached 10- 15 min after uptake. The last endocytotic compartment, the lysosome, is further acidified to pH values of 5.0 or lower and is reached 20 min or more after uptake. The lysosomes are the main degrading compartment in the endocytotic pathway. Conventional, pH-insensitive liposomes and their content are delivered to lysosomes and degraded. The last requirement for plasmid liposomes after cell penetration is to avoid accumulation in particular cell compartments such as lysosomes. In order to prevent this degradation, pH-sensitive liposomes have been proposed. pH-sensitive liposomes were designed based on the concept of viruses that fuse with the endosomal membrane by means of protein at pH 5-6, delivering their genetic material to the cytosol before reaching the lysosomes. Generally, the lipid used to design pH sensitive liposomes is PE. PE represents a lass of lipids which, when dispersed in pure form, assemble into nonbilayer structures in n inverted hexagonal phase.To stabilize PE in the lamellar hase in liposomes a series of stabilizers possessing titratable acid headgroup such as oleic acid (OA), palmitoylhomocysteine PHC) and cholesterolhemisuccinate CHEMS) were used. Liposomes composed of DOPE/OA/chol are capable of transfecting mouse Ltk-cells cells lacking thymidine kinase (TK) with n exogenous TK gene. In this study, pHsensitive iposomes were 8-fold more efficient in gene delivery than pH-insensitive liposomes. Interestingly, the same investigators also demonstrated that plasmid DNA adsorbed to preformed empty pH-sensitive liposomes can transfect murine Ltk-cells in vitro. In contrast, negligible transfection by free plasmid DNA was observed. In a study by Zhou et al.,pUCSV2 CAT DNA was used to prepare liposomes composed of DOPE/dioleylsuccinyl glycerol (DOSG) (pH-sensitive formulation) or of dioleylphosphatidylcholine/DOSG (pH-insensitive formulation). The data showed that the acid sensitivity was directly related to the transfection activity. DOPE/DOSG liposome, which was the most sensitive to pH, transfected cells with the highest efficiency. Legendre and Szoka compared the transfection efficiency mediated by pH-sensitive, pH-insensitive and cationic (DOPE/ dioleyloxypropyl-trimethylammonium bromide (DOTMA) liposomes using two different genes and five different cell lines. For all cell types investigated, cationic liposomes mediated the highest transfection level. While pH-sensitive liposomes mediated gene transfer, their efficiency was 1-30% of that obtained with DOPE/DOTMA and pH-insensitive liposomes did not induce transfection. It is important to emphasize the fact that separation of nonencapsulated (adsorbed or free) DNA was performed by pHsensitive but not by cationic liposomes. This fact itself may interfere with the performance of the two types of liposomes. They suggested that the greater activity of oligonucleotides encapsulated into pH-sensitive liposomes was not due to a destabilization of the DOPE liposome bilayer but to an increased association between pH-sensitive liposomes and cells. They reported that the efficiency of the viral inhibition obtained with oligonucleotides encapsulated into pHsensitive liposomes was only twice that of oligonucleotides encapsulated into non-pH-sensitive liposomes. And a two-fold increase in cell association was also observed when pH-sensitive liposomes were compared to pH-insensitive liposomes. In fact, pHsensitive liposomes are taken up more efficiently by cells than pH insensitive liposomes, a fact probably leading to a better activity.

***Cationic Lipid Strategy***

The encapsulation of DNA into conventional liposomes could be a technical problem due to the plasmid size, representing a poor transfection system. On this basis, an alternative technology based on cationic lipids and PE was developed in the late 1980s. The idea was to neutralize the negative charge of plasmids with positively charged lipids to capture plasmids more efficiently and to deliver DNA into the cells. Generally, this is a simple procedure requiring mixing the cationic lipids with the DNA and adding them to the cells. This results in the formation of

aggregates composed of DNA and cationic lipids. The cationic lipid DOTMA was first synthesized and described by Felgner et al. This lipid, either alone or in combination with other neutral lipids, spontaneously forms multilamellar vesicles (MLV) which may be sonicated to form small unilamellar vesicles (SUV). DNA interacts spontaneously with DOTMA to form DNA complexes with 100% of the DNA becoming associated. It is presumed that complex formation simply results from ionic interactions between the positively charged headgroup of DOTMA and the negatively charged phosphate groups of DNA. DOTMA is commercialized (Lipofectin., Gibco-BRL, Gaithersburg, MD) as a one to one mixture with DOPE and has been widely used to transfect a wide variety of cells. In an effort to reduce the cytotoxicity of DOTMA, a series of metabolizable quaternary ammonium salts have been developed whose efficiency is comparable to that of Lipofectin when dispersed with DOPE. As stated in the list of requirements, one important step for transfection is DNA compaction to improve cell penetration. Cationic amphiphiles able to compact genomic DNA, namely lipopolyamines, have been studied. Among them, DOSG (Transfectam.) has been shown to transfect many animal cells in a highly efficient manner.These amphiphiles have been shown to stably condense DNA into particles. Common detergents of diverse structures (cetyl-trimethylammonium bromide (CTAB), dodecyltrimethylammonium bromide (DDTAB) have been compared for use in combination with DOPE. DDTAB seemed to be the most promising one and the DTAB/DOPE formulation was patented (TransfectACE.). As reported by Farhood et al., the role of DOPE in cationic liposome-mediated gene transfer seemed to be critical, and the compound has been extensively used. Since it has been postulated that the mechanism of DNA/cationic lipids uptake by cells is related to endocytosis, DOPE may favor the liberation of DNA into the cytosol as in pH-sensitive formulations. Electron microscopy observations have shown the endosome destabilizing effect of DOPE-containing cationic liposomes, although efforts to synthesize new cationic lipids led to the discovery of more efficient transfection agents, their efficiency does not correlate with their ability to deliver DNA after systemic administration to animals. The physicochemical properties of the DNA/lipid complex may determine its stability in plasma and its biodistribution or pharmacokinetics. In an effort to determine the physicochemical properties of the complex, cationic lipids associated with DOPE and with various amounts of three different cationic surfactants have been investigated by cryo-transmission electron microscopy (TEM). cyo-TEM analysis suggests that an excess of lipids in terms of charge leads to entrapment f the DNA molecules between the lamellae in clusters of aggregated multilamellar structures. The choice of surfactant does not appear to affect the morphology of the DNA-lipid-complexes. Furthermore, the system containing DOPE results in more compact aggregates than similar formulations using egg lecithin. Templeton et al. have proposed a model for the assembly of DNA-lipid (N-1(2,3-dioleyloxy) propyl, N,N,N-trimethyl ammonium methyl sulfate- DOTAP)-chol complexes in which DNA adsorbs onto the invaginated and tubular liposomes via electrostatic interactions. This generates closed structures in which DNA may be protected. Farhood et al. [48] proposed the endocytosis s the major route for DNA-lipid complex uptake by cells during transfection. The surface-bound complex is internalized by endocytosis into endosomes and lysosomes n which a large part of the DNA would be degraded. According to Hui and Zhao, the most evident pathway for DNA entry into CHO cells is also endocytosis and not direct fusion of the complex with the plasma membrane. Once inside the cell, how and then DNA and lipids become separate remains in question.

***Liposome for Targeted Delivery [6,7]***

Use of liposome-encapsulated enzymes for delivery into cells was first reported in 1971. About the same time, a specific receptor on hepatocytes was demonstrated to mediate clearance of ß-galactose–terminated glycoproteins from circulation. A

mannoside-specific receptor was recognized on the cell surface of the RES of rats (including the liver sinusiod and macrophages). By grafting different glycosides on the surface of liposomes, it is possible to direct the latter to different cell types of rat liver. Galactosylated liposomes are mainly taken up by liver hepatocytes, whereas mannosylated liposomes are mainly taken up by nonparenchymal cells. Grafting specific ligands to the liposome surface facilitates a fusion of the liposome with target cells by endocytosis, thus releasing material to be delivered. In cancer chemotherapy, the toxicity of anticancer drugs is of major concern. Liposomes could be used to deliver such drugs and minimize their toxic effects on healthy cells. Targeted delivery to cancer cells could be achieved by coating monoclonal antibodies (MAbs) raised against tumor-cell specific antigens. In vitro and in vivo studies by Ahmad et al. of squamous-cell carcinoma in mouse models provided evidence that antibody-coated polyethyleneglycol liposomes containing doxorubicin were more effective and less toxic than free drugs, drugs incorporated into antibody-free liposomes, and antibodycoated conventional liposomes. The major concern in antibody-grafted liposome use is the induction of immune response to the grafted antibodies. Basten et al. suggested a novel approach to overcoming that difficulty.[4] They used 125I-labeled antigen to kill the cells responsible for immune induction (the "antigen suicide" technique).

Other possible approaches to overcome the immune-system problem include immunosuppressive drugs and humanized antibodies or establishing neutral immune windows for subsequent injection. Liposomes can be designed to release their entrapped contents under certain controlled conditions: pH-sensitive and temperature-dependent liposomal systems.[6,7] Drug targeting using liposomes as carriers holds much promise, especially in reducing toxicity and targeting delivery to disease sites. The future is bright for liposome research, with a large number of clinical trials ongoing in several countries with liposomal formulations of various anticancer drugs, antisense, cytokines, peptides and proteins. In the near future, several more liposome-based drugs will find their way into the pharmaceutical market.

***Liposomes In Cosmetics***

In the past, the beauty enhancements expected from cosmetic products were obtained simply by combining the moisturizing, bleaching, or cell generating agents with the cosmetic base. Now cosmetic products have reached the stage where liposomes can encapsulate active ingredients thought to be necessary for the skin so they may be directly applied to the skin cells. Most useful for being able to transfer and deliver active ingredients to the application site of cosmetics. The liposome wall is very similar, physiologically, to the material of cell membranes. When cosmetic containing liposomes is applied to the skin, for example, the liposomes are deposited on the skin and begin to merge with the cellular membranes. In the process, the liposomes release their payload of active materials into the cells. As a consequence, not only is delivery of the actives very specific directly into the intended cells but the delivery takes place over a longer period of time. Liposomes are typically manufactured from various fatty substances that are used to encapsulate, or to create a sphere around, cosmetic materials. They act as a delivery system. Today, most of the experts working in the field of liposomal dispersions agree that liposomes do not penetrate as intact vesicles into the skin or permeate through the skin. Liposomes are believed to be deformed and transformed into fragments as a rule. Therefore size, shape, and lamallarity are not so relevant for the application, but for the chemical composition of the total formulation. The multifunctional properties of phosphatidylcholines lead to a number of different applications. So, formulations with unsaturated phosphatidylcholine are preferred to support skin regeneration, antiaging, acne preventing, and penetrating other active agents like vitamins and their derivatives into the skin. Formulations with hydrogenated phosphatidylcholine may be used for skin and sun protection, but it should be emphasized that in this respect nanoparticles and DMS are still more convenient. The numerous patents

on liposome applications reflect the avid interest of many companies in this area. A patent involves a skin whitening lotion in which liposomes, consisting of vitamin E and complex lipids are dispersed in alcohol and water. The patent claims that vitamin E remains stable long enough to exhibit reducing action. A second patent related to skin whitening cosmetics involves the use of liposomes encapsulating ascorbic acid and alpha-tocopherol (vitamin E). This patent claims that the use of liposomes prevents the oxidation of ascorbic acid. The study showed that decomposition of ascorbic acid was inhibited by the use of liposomes. One patent claims that the stability of a liposome is enhanced by covering its surface with fatty acid esters and polysaccharides. The next patent concerns a process for manufacturing large quantities of liposomes. Dissolving an amphiphilic substance in a solvent and spray drying the resulting solution gives rise to a fine powdery mixture.[9,10] Thispowder, when dispersed in an aqueous medium and mixed, easily yields large quantities of liposomes. Phosphatidylserine, sphingomyelin or soybean lecithin may be used as the amphiphilic components, and yeast, antibiotics, elastin polypeptides, aloe vera etc., may be used as encapsulated active ingredients. Another patent claims that a membrane is formed by dissolving lecithin or phosphatidylcholine in an organic solvent which is then evaporated. The homogenization of this membrane with active ingredients can yield liposomes containing active ingredients which can be used in the manufacture of cosmetics. The patent also confirms the percutaneous absorption of the liposome's active ingredients through the use of a [14] C label. Liposomal dispersions have proved not only to be innovative and effective cosmetic ingredients, but also to be a very convenient form to work with phosphatidylcholine. In dermatology, they will be used with success for preventing and treating several skin diseases. Complementary formulations are established where liposomal dispersions come up against limiting factors. Generally, members of the membrane family like liposomes, nanoparticles, and DMS are more compatible with the skin structure than usually applied conventional emulsions. "Compatible" means that formulations do not disturb the integrity of the skin lipid bilayers and are not washed out when the skin is cleaned. In the sense of modern strategies of cosmetics, these formulations get by with a minimum of auxiliary compounds, which put only a strain on the skin. Moreover, compatibility means embedding lipids and hydrophilic agents in the horny layer and being in line with the natural situation. Remarkably, phosphatidylcholine need not be applied in high concentrations, because experience shows that formulations are stable at lower amounts. Also, there is a cumulative effect in the horny layer with repeated application of phosphatidylcholine. In many cases liposomes, nanoparticles and DMS are compatible with each other in a sense that they can be used as a modular system. So these formulations are believed to still have a great future in cosmetic science. How far new findings about the importance of the choline moiety of phosphatidylcholine [10] will impact skincare research and development cannot be estimated.

### *2) Application of Liposome*

The field of liposome research has expanded considerably over last 30 years. It is now possible to engineer a wide range of liposome of varying size, phospholipids composition, cholesterol composition, surface morphology suitable for wide range of application. Liposomes interact with cells in many ways to cause liposomal components to be associated with target cells. The liposome carrier can be targeted to liver and spleen and distinction can be made between normal and tumors tissue using tomography. In case of transdermal drug delivery system, liposome has a great application. Liposomal drug delivery system when used to target the tumor cells leads to reduction in the toxic effect and enhances the effectiveness of drugs. The targeting of the liposome to the site of action takes place by the attachment of amino acid fragment, such as antibody or protein or appropriate fragments that target specific receptors cell. Liposomal DNA delivery vectors and further enhancement in the form of LPDI-Iand LPD-II are some of the safest and potential most

versatile transfer vectors which are used to date. DNA vaccination and improved efficiency of gene therapy are just a few of the recent application of liposome. Several modes of drug delivery application have been purposed for the liposomal drug delivery system, few of them are as follows:

- Enhance drug solublisation (Amphotericin-B, Minoxidil, Paclitaxels, and Cyclosporins)
- Protection of sensitive drug molecules (Cytosine arabinosa, DNA, RNA, Anti-sense oligonucleotides, Ribozymes)
- Enhance intracellular uptake (Anticancer, anti viral and antimicrobial drugs)
- Altered pharmacokinetics and bio-distribution (prolonged or sustained released drugs with short circulatory half life) Several recent applications of liposomal drug delivery system are as follows:

***A. Liposome for Respiratory Drug Delivery System***

Liposome is widely used in several types of respiratory disorders. Liposomal aerosol has several advantages over ordinary aerosol which are as follows:

1. Sustained release
2. Prevention of local irritation
3. Reduced toxicity and
4. Improved stability in the large aqueous core.

Several injectable liposome based product are now in the market including ambisome, Fungisome and Myocet. To be effective, liposomal drug delivery system for the lung is dependent on the following parameters:

1. Lipid composition
2. Size
3. Charge
4. Drug and Lipid ratio and
5. Method of delivery

The recent use of liposome for the delivery of DNA to the lung means that a greater understanding of their use in macromolecular delivery via inhalational is now emerging.
Much of this new knowledge, including new lipids and analytical techniques, can be used in the development of liposome based protein formulations.
For inhalation of liposome the liquid or dry form is taken and the drug release occurs during
nebulization. Drug powder liposome has been produced by milling or by spry drying

***B. Liposome in Nucleic Acid Therapy***

Recombinant DNA technologies and studies of gene function and gene therapy all depends on the successful delivery of nucleic acid into cells *in vitro* and *in vivo*. Non-viral vectors will be developed for the selective delivery of the gene to the malignant cells. The vector will exploit the increase requirements of rapidly growing cells for more nutrients by attaching a nutrients ligand onto the vector (liposome). The vector additionally will have a passively charged lipid to enhance nucleic acid binding along with novel pH[12].

***C. Liposome in Eye Disorders***

Liposome has been widely used to treat disorder of both anterior and posterior segment. The disease of eye includes dry eyes, keratitis, corneal transplant rejection, uveitis, endopthelmitis
and proliferative vitro retinopathy. Retinal diseases are leading cause of blindness in advanced countries. Liposome is used as vector for genetic transfection and monoclonal antibody directed vehicle. The recent techniques of the treatment like applying of focal laser to heat induced elease of liposomal drugs and dyes are used in the treatment of selective tumour and neo-vascular vessels occlusion, angiography, retinal and choroidal blood vessel stasis. Liposomal drug formulations have been approved for the two of patent drugs to date and several other products are under clinical trials.[13-15].

***Future prospectus[5]***

This approach is necessary because the use of liposomes for drug delivery in the future will depend on the liposome-drug formulation having clear advantages over the conventional use of the therapeutic agent not in the lcast

facing comparatively higher cost of production. Especially the liposome-cell interaction. the transport mechanisms across the stratum corneum, the role of the transfollicular pathway and the liposomeliposome interaction must be investigated in greater detail. Moreover, one must take Hypoallergenic, fragrance-free, paraben-free, contains no animal products, no mineral oil, no artificial colors, and no artificial or harmful preservatives. Smoothes into the skin easily and helps to restore a lustrous, healthy looking sheen to the skin. A closer look at the behaviour of drugloaded liposomes in healthy and diseased skin in vitro. In this context, especially skin diseases with a defect of the permeability barrier seem to be interesting.While intact liposomes are said to penetrate only the superficial parts of the living epidermis this might not hold true in diseased skin. For example, in psoriasis vulgaris, topically applied drugs encapsulated into liposomes might reach lower strata of the epidermis or even the dermis. For this reason recently reported experimental data about topical liposomal drugs (liposomal xanthines, psoralens and dithranol) in psoriasis- therapy warrant further interest. Another promising held for therapeutical progress with topical liposome drugs is UV-induced skin cancer: Yarosh et al. investigated the effect of liposomes containing DNA repair enzymes in vitro and in vivo (animal experiments). They found that liposomes can deliver encapsulated proteins into cells of the skin leading to a reduction of UV-induced skin cancer in mice.

## CONCLUSION

It was concluded from the review that liposomes have great potency in drug delivery system. Drug of both category (hydrophilic/ lipophillic) easily embedded in the liposomes. The drug was delivered in the body in the controlled manner or wants to be site specific. Liposomes have developed into a viable pharmaceutical dosage form. Progress has taken place in quantum leaps, rather than in a continuum, over the last two decades. Vital progess have been made in the development of long circulating liposomes that are not immediately recognized and removed by the cells of mononuclear phagocyte system. Despit this long circulating liposomes have opned a new realm of therapeutic opportuities and we will see a multitude of novel applications emerge in future. Development will continue to explore the validity of liposomes for the delivery of peptide and proteins, although progress in thid particular field has been meager. These developments will hopefully safeguard against the overoptimistic and unrealistic ideas and promises of the past and lead into another highly productive and innovative phase of liposome research.

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