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REVIEW ARTICLE

A Review on Liposomes

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ABSTRACT

Basically liposomes are resultant of self assembly of phospholipid molecules in an aqueous environment. The amphiphilic phospholipid molecules from a closed bilayer sphere in an attempt to shield their hydrophobic groups from the aqueous environment while still maintaining contact with the aqueous phase via the hydrophilic head group. The resulting closed sphere may encapsulate aqueous soluble drugs within the central aqueous compartment or lipid soluble drugs within the bilayer membrane. Alternatively lipid soluble drugs may be complexed with cyclodextrin and subsequently encapsulated within the liposomes aqueous compartment. The encapsulation within association of drugs with liposomes may alter drug pharmacokinetics, which may be exploited to achieve targeted therapies. Thus alteration of the liposomes surfaces and their further modification are some of the requisites of optimized liposomal drug targeting Preformulation of drugs in liposomes has provided an opportunity to enhance the therapeutic indices of various agents mainly through alteration in their biodistribution. This review discusses the potential applications of liposomes in drug delivery with examples of formulations approved for clinical use, and the problems associated with further exploitation of this drug delivery system.

Key words: Liposomes, Phospholipid, Encapsulation, Applications

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INTRODUCTION

Liposomes have been receiving a lot of interest as a carrier for advanced drug delivery. Liposomes were first produced in England in 1961 by Alec D. Bangham, who was studying phospholipids and blood clotting [1]. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule is water soluble, while the opposite end is water insoluble. Water soluble medications added to the water were trapped inside the aggregation of hydrophobic ends fat-soluble medications were incorporated into the phospholipid layer. A liposome is a spherical vesicle with a membrane composed of a phospholipid bilayer used to deliver drug or genetic material into a cell. Liposomes can be composed of naturally-derived phospholipids with mixed lipid chain like egg phosphatidylethonalimine or of pure components like DOPE (dioleolyl phospha tid ylethano lamine). The lipid bilayer can fuse with other bilayers eg. The cell membrane Fig. 2, thus delivering the liposome contents. By making liposomes in a solution of DNA or drugs, (which would normally be unable to diffuse through the membrane), they can be delivered past the lipid bilayer [2]. The use of liposomes for transformation or transfect ion of DNA into a host cell is known as lipofection. Liposomes can be created by sonicating phospholipids in water. Low shear rates create multilamellar liposomes, which have many layers like an onion. Continued high-shear sonication tends to form smaller unilamellar liposomes. Liposome is a Spherical vesicles with a phospholipid bilayer

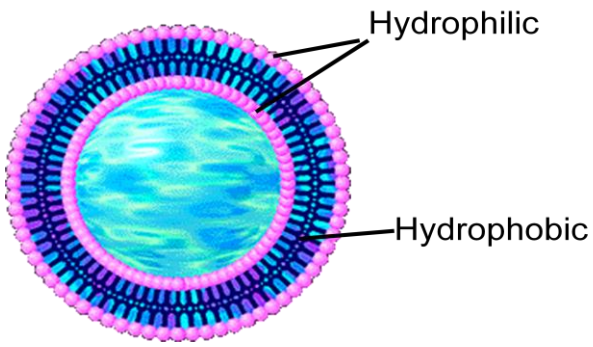

Fig.1 Phospholipids

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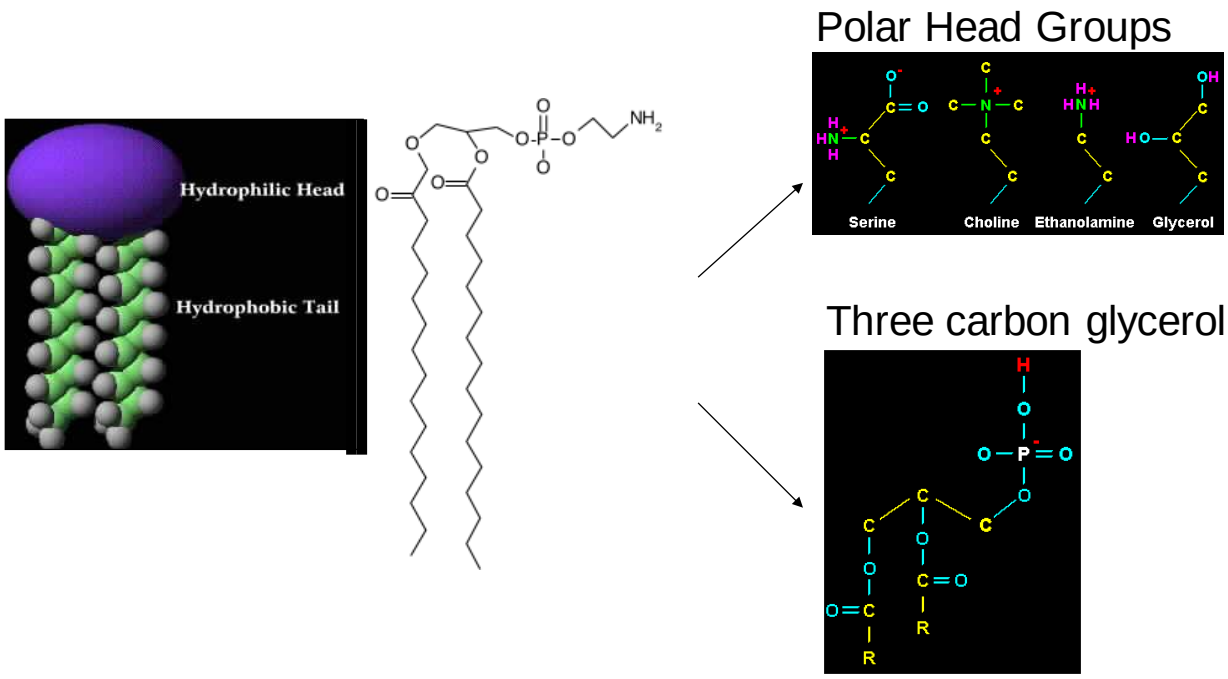

Fig.2 Cell Membrane

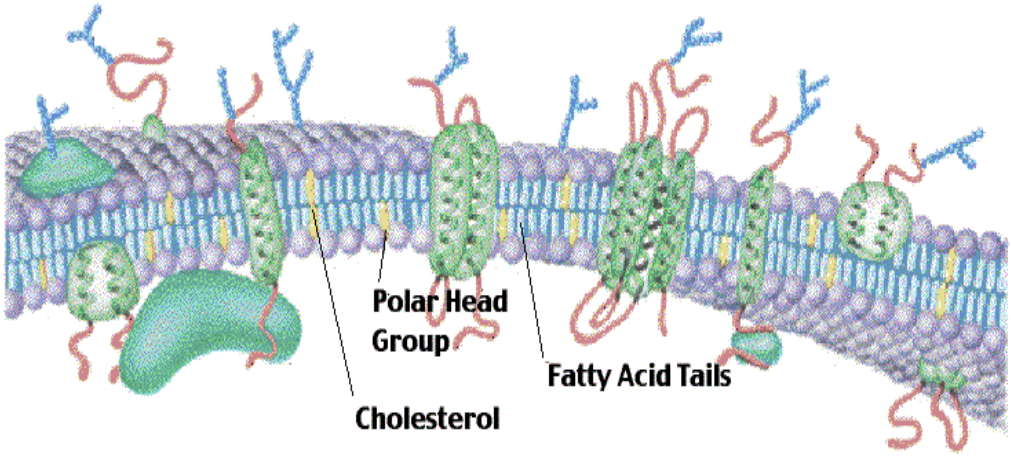

Fig.3 Structure of lipids

Uses of Liposomes

- Chelation therapy for treatment of heavy metal poisoning
- Enzyme replacement
- Diagnostic imaging of tumors
- Cosmetics

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- Study of membranes

Liposomes in Drug Delivery

Drug Targeting

- Inactive: Unmodified liposomes gather in specific tissue reticuloendothelial system
- Active: alter liposome surface with ligand (antibodies, enzymes, protein A, sugars)
- Physical: temperature or pH sensitive liposomes
- Directly to site

Pharmokinetics efficacy and toxicity

- Changes the absorbance and biodistribution
- Deliver drug in desired form
- Multidrug resistance
- Protection
- Decrease harmful side effects
- Change where drug accumulates in the body

TYPE OF LIPOSOMES

Depending upon the structure there are two type of liposomes.

a) Unilamellar liposomes [3]: Unilamellar vesicles has a single phospho-lipid bilayer sphere enclosing aqueous solution as shown in Fig. 5.

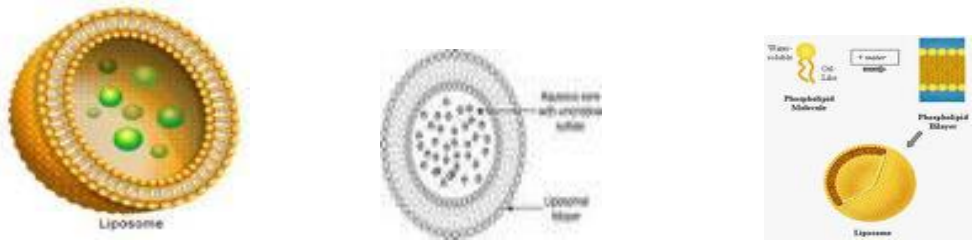

Fig.5 Very Small, Single Layer liposome

b) Multilamellar Liposomes [4]: Multilamellar vesicles have onion structure. Typically, several Unilamellar vesicles will form one inside the other in diminishing size, creating a multilamellar structure of concentric phospholipid spheres separated by layers of water as shown in Fig 6.

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Fig.6 Large Vesicle, Multilayer Liposome

COMPOSITION AND CHARACTERSTICS OF LIPOSOMES

Usually liposomes composed of cholesterol and phospholipids. The structure, composition and proportion being practically the same as in the host cell membranes. The phospholipids possess a hydrophobic tail structure and a hydrophilic head component and organize in the following when dissolved in water, the hydrophobic tails mutually attract while the hydrophilic heads contact with the aqueous medium external and internal to the liposome surface[5]. In this way, double lipid layers are formed which seal off to form small vesicles similar to the body cells and organelles. These sphere or liposomes constitute small deposits that can be made to contain an antigen, an antibiotic, an allergen, a drug substance a gene. The liposomes can in turn be introduced in the body without triggering immune rejection reaction. Phospholipid bilayers are the core structure of liposomes and cell membrane formation.

MECHANISM OF TRANSPORTATION THROUGH LIPOSOMES [6]

Liposome can interact with cells by four different mechanism

- Endocytosis by phagocytic cells of the reticuloendothelial sys-tem such as macrophages and neutrophils.
- Adsorption to the cell surface either by nonspecific weak hydro-phobic or electrostatic forces or by specific interactions with cell-surface components.
- Fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal content into the cytoplasm
- Transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents.
- It often is difficult to determine what mechanism is operative and more than one may operate at the same time as shown in Fig. 7.

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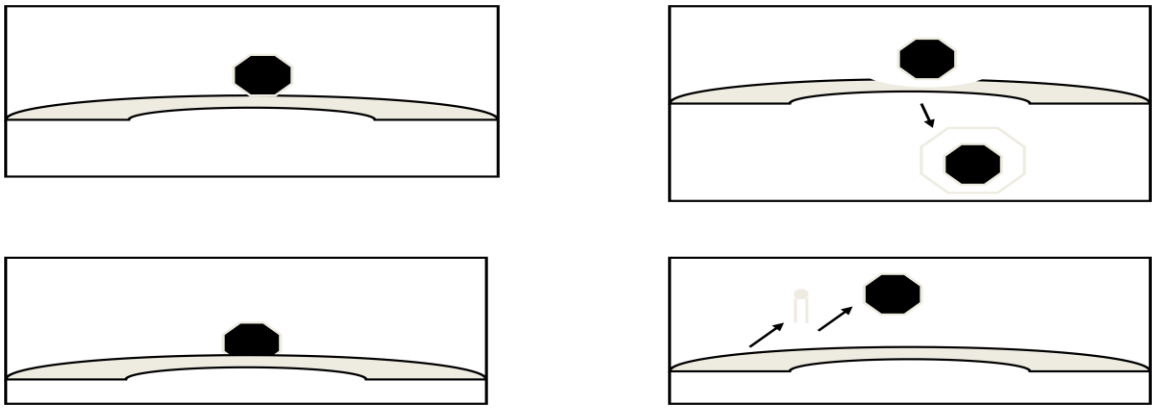

Fig. 7 Mechanism of Transportation Through Liposomes

LIPOSOME PREPARATION

a) Handshaking Method [7]

In order to produce liposome lipid molecules must be introduced into an aqueous environment. When dry lipid layer film is hydrated the lamellae swell and grow into myelin figures. Only mechanical agitation provided by vortexing, shaking, swering or pippeting causes myelin figures to break and reseal the exposed hydrophobic edges resulting in the formation of liposomes can be made by hand shaken method.

b) Sonication Method [7]

This method is probably the most widely used method for the preparation of small Unilamellar vesicles. There are two sonication techniques:

i) Probe Sonication

The tip of sonicator is directly immersed into the liposome dispersion is very high in this method. The dissipation of energy at the tip results in local overheating and therefore the vessel must be immersed into an ice bath. During the sonication up to one hour more than 5% of the lipids can be desterify. Also, with the probe sonicator, titanium will slough off and contaminate the solution.

ii) Bath Sonicator

The liposome dispersion in a tube is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method compare to sonication the dispersion directly using tip. Material being sonicated can be kept in a sterile container, unlike the probe units, or under an inert atmosphere. The lipid bilayer of the liposomes can fuse with other bilayers, thus delivering the liposome contents. By making liposomes in a solution of DNA or drug they can be delivered past lipid bilayer.

c) Reverse Phase Evaporation Method [7]

Historically this method provided a breakthrough in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid ratio and able to entrap a large percentage of the aqueous material presented. Reverse phase evaporation is based on the formation of inverted micelles. These inverted micelles are formed upon sonication of a mixture of a buffered aqueous phase, which contains the water soluble molecules to be encapsulated into the liposomes and an organic phase in which the amphiphilic molecules are solubilized. The slow removal of the organic solvent leads to transformation of these inverted micelles into a gel like and viscous state. At a critical point in this procedure, the gel state collapse and some of the inverted micelles into a gel like and viscous state. At a critical point in this procedure, the gel state collapse and some of the inverted micelles disintegrate. The excess of phospholipids in the environment contributes to the formation of a complete bilayer around the remaining micelles, which results in formation of liposomes. Liposome made by this method can be made from various lipid formulations and have aqueous volume to lipid ratios that are four time higher than multi lamellar liposomes or hand shaken method.

d) Freeze Dried Rehydration Method [7]

Q Freeze dried liposomes are formed from preformed liposomes. The very high encapsulation efficiencies even for macromolecules can be achieved using this method. During the dehydration the lipid bilayers and the material to be encapsulated into the liposomes are brought into close contact. Upon swelling the chances for encapsulation of the adhered molecules are much higher. The rehydration is a very important step and is should be done very carefully. The aqueous phase should be added in very small portions with a micropipette to the dried materials. After each addition the tube should be vortexed thoroughly. As a general rule the total volume used for rehydration must be smaller than the starting volume of the liposome dispersion.

FORMULATION OF LIPOSOME

Liposomes are made from pure lipids or a combination of lipids. The lipids commonly employed in liposome formulations are phospholipids [8]. Liposomes have been prepared from a variety of synthetic and naturally occurring phospholipids, they generally contain cholesterol. The incorporation of cholesterol into the lipid bilayer membrane generally enhances the stability of liposomes in serum, reduces the permeability of the membranes to water soluble molecules and increases the fluidity or microviscosity of the bilayer usually, a zwitter ionic or non ionic lipid is used as the basic lipid for the preparation of liposomes. The net surface charge of liposome can be modified by the incorporation of positively charged lipids such as stearylamine, or negatively charged lipids such as diacetylphosphate, phosphatidyl glycerol or phosphatidyl serine. The presence of negatively or positively charged lipids lead to a greater overall volume for aqueous entrapment and reduces the likelihood of aggregation after preparation of the liposomes. Cationic liposomes display some disadvantages such as cytolytic

and cytotoxic activities. Yoshihara and Nakae have demonstrated that cationic liposomes containing stearylamine showed an in vivo toxicity in rabbits. This effect was attributed to haemolysis of the erythrocytes and was directly related to the amount of stearylamine present in the liposome composition.

Formulation factors affecting the degree of drug entrapment

The extents of drug entrapment and retention as well as factors influencing them are important considerations in the design of liposome mediated drug delivery systems. Drugs may be entrapped in the aqueous and lipid phase of the liposome.

a) Aqueous entrapment

This relates to the aqueous volume in the liposome. The larger the aqueous volume the greater the amounts of polar drugs that can be encapsulated [9]. Multiple compartment liposomes encapsulate higher percentages of aqueous soluble drugs than single compartment vesicles, because of the larger volume of encapsulated aqueous space in the former. Formulations that promote formation of MLVs are thus associated with higher aqueous entrapment. Osmotic swelling and incorporation of charged lipids, e.g., phosphatidylserine into bilayers are measures for increasing the aqueous volume in liposomes. The latter is due to charge repulsion separating adjacent bilayers, resulting in increases in trapped aqueous volume. Aqueous solubility of the drug is another factor, hence, the extent of drug entrapment in liposomes (MLVs) can vary markedly as seen in the following examples: 2.2–8.4% for penicillin, 11.6% for actinomycin D, 18% for methothrexate and up to 60% for bleomycin. Leakage of entrapped solute is another formulation problem. Cholesterol modifies the fluidity of lipid membranes, thereby influencing the degree of retention of drugs by vesicles as well as stabilizing the system against enzymatic degradation. Large molecules (e.g., peptides and proteins) are better retained than smaller molecules, which can diffuse slowly through the lipid layers.

b) Lipid entrapment

Lipid soluble drugs are entrapped in the lipid layers of liposome. Here, the entrapment efficiency can be as high as 100%, irrespective of liposomal type and composition. An example of a drug that is hydrophobic in nature is camptothecin [10]. The retention of such hydrophobic drugs is also high when the liposomes are placed in aqueous biological environment because of their high lipid-water partition coefficients.

Formulation factors affecting stability of liposomes

The stability of liposomes refers to their ability to retain entrapped solutes, chemical stability of both the entrapped solutes and the lipid membranes. Solute leakage depends on membrane permeability and on the interaction with components of biological fluids.

Membrane fluidity can be controlled to reduce leakage by supplementing the lipid bilayer with cholesterol or by manipulating the hydrophobic character of the bilayers, for example, with the use of fluorinated lipid. The rate of solute leakage also depends on the lamellar structure of liposomes, for instance, MLVs are less prone to leakage than ULVs. In order to minimize leakages liposomes are stored in the form of freeze-dried powders [11]. The lipid vesicles can undergo chemical degradation. For instance, phospholipids can be hydrolysed to lysophospholipids, which are also subject to further hydrolysis. The lysophospholipids are the main initial products of hydrolysis. Hydrolytic degradation of either the lipid or entrapped drug may be pH related but can be prevented or minimised by freeze drying of liposome suspension to dry powders. When unsatu-rated phospholipids are used to prepare liposomes, special precautions must be taken to minimise oxidation. These include the use of light resistant containers, use of antioxidants such as α-tocophenol, deaeration with argon or nitrogen to minimise exposure to oxygen, and removal of heavy metals from the preparation.

Storage of liposomes [12]

Liposome dispersions are potentially prone to hydrolytic degradation and leakage. Hence, it is desirable to freeze dry the suspension to a powder and store in this dried form. The powder can be reconstituted to an aqueous suspension immediately before use. By doing so SUVs may be converted to MLVs dispersion upon rehydration. Addition of a carbohydrate (trehalose) during freeze drying prevents fusion and leakage of the vesicles.

Pharmacokinetic considerations

Most small molecular chemotherapeutic agents have a large volume of distribution on intravenous (IV) administration of liposomes. The result of this wide distribution is often a narrow therapeutic index due to a high level of toxicity on healthy tissues. Through encapsulation of drugs in liposomes, the volume of distribution is significantly reduced and the concentration of drug at the desired site of action increased [13]. For instance, liposomal drug delivery led to an increase in the amount of drug that can be effectively delivered to tumour sites in anticancer therapy. Liposomes are predominantly removed from circulation by phagocyte cells of the reticuloendothelial system (RES), thus accumulating to a large extent in organs like liver and spleen. This biodistribution pattern can be used for passive targeting of diagnostics to these organs. The RES should therefore be saturated with empty vesicles when other sites are the drug targets. Information on biodistribution is therefore important for drug targeting by liposomes. Liposomes given intravenously usually interact with at least two distinct groups of plasma proteins. These are the plasma high density lipoproteins and the so called opsonins, which bind to the surface of vesicles and mediate their endocytosis by the mononuclear phagocyte system (macrophages). The rate of liposome clearance from blood circulation will, therefore, depend on the ability of opsonins to bind to the liposome surface [14]. The rate can be manipulated through appropriate selection of liposome characteristics. For instance, “fluid” vesicles are removed more rapidly from blood circulation than “rigid” ones. Clearance from the blood stream is also influenced by vesicle size and surface charges. The

longest half life is obtained when liposomes are relatively small (diameter <0.05.m) and carry no net surface charge. The pharmacokinetic behaviour of liposomes depends on the route of injection such as intraperitoneal, subcutaneous or intramuscular route.

Biomedical applications of liposomes

Both hydrophilic and hydrophobic drugs can be encapsulated in liposomes. Liposomes are also relatively non-toxic and biodegradable [15]. They therefore have a wide range of biomedical applications.

Protection against enzymatic degradation of drugs

Liposomes are used to protect the entrapped drug against enzymatic degradation while in circulation. The basis is that the lipids used in their formulation are not susceptible to enzymatic degradation the entrapped drug is thus protected while the lipid vesicles are in circulation in the extracellular fluid upon penetration into the cell, the entrapped drug is released either by diffusion through the microsphere shell, dissolution of the shell or degradation of the shell by lysosomal enzymes. Thus β-lactamase sensitive antibiotics, e.g., the penicillins and cephalosporins have been encapsulated due to this reason to protect against the β - lactamase enzyme. Liposomes also offer protection for its encapsulated drugs in the environment of the gastrointestinal tract and facilitate the gastrointestinal transport of a variety of compounds. Liposomes are therefore candidates to be explored for oral delivery of insulin and proteins for use as vaccines, which are otherwise orally degradable [16]. Liposomes offer a number of advantages as carriers of vaccine agents in that they are biodegradable and non toxic. Drugs encapsulated in lipososmes can elicit both humoral immunity when given orally and cell mediated immunity. Liposomes are now being employed as oral vaccines in numerous immunization procedures. Twenty five years after the discovery of the immunological adjuvant properties of liposomes, they are now considered the major candidate as the base for oral vaccine against hepatitis A, which is being licensed for use in humans.

Drug targeting

The need for “drug targeting” arises from a problem situation whereby a drug administered (iv for example) enters the blood stream and is distributed to varying extents throughout the body when the actual desire is to deliver or direct the drug selectively to its site of action. This site could be an organ structure, a cell subset, or even an intra cellular region. In such a case pumping the drug throughout the whole body is not only wasteful but, more fundamentally, it is also likely to lead to undesirable side effects. On the other hand, restricting the distribution of the drug to the specific target site should allow for an increase in efficacy at low dose with attendant decrease in toxicity. Hence, the benefits of drug targeting include reduced drug waste, and it is possible to deliver a drug to a tissue or cell region not normally accessible to the free or untargeted drug. The approach for drug targeting via liposomes involves the use of ligands (e.g., antibodies, sugar residues, apoproteins or hormones), which

are tagged on the lipid vesicles. The ligand recognises specific receptor sites and, thus, causes the lipid vesicles to concentrate at such target sites [17]. By this approach the otherwise preferential distribution of liposomes into the reticuloendeothelial system RES (liver, spleen and bone marrow) is averted or minimised. The preferential distribution of liposomes into the RES can be modified by the incorporation in the liposome membrane of protein or carbohydrates possessing specific affinity toward a target tissue or organ. A ligand selection is based on its recognition by, and specificity for, the target site. In cancer treatment, for example, one of the approaches is to target the drug to tumour cells via receptor specific ligands, which may be specific antibodies for antigens produced by the tumour cells. The first step, therefore, is to determine the antigens that are produced by the tumour cells. Also, molecules bearing oligosaccharide chains have been used as ligands for direction, and specific attachment, to ganglion sites in cells.

Topical drug delivery

The application of liposomes on the skin surface has been proven to be effective in drug delivery into the skin. Liposomes increase the permeability of skin for various entrapped drugs and at the same time diminish the side effect of these drugs because lower doses are now required. Liposomes have also found an important application in cosmetics for skin care preparations. In this regard, the liposomes are applied to the skin in the form of solution or in hydrogels. Hydrophilic polymers are suitable thickening agents for the gels [18]. However, the liposomes may in certain instances be trapped in the polymeric network of the hydrogels and, hence, impair bioavailability into the skin. Nevertheless, It found enhanced transport of liposome entrapped substances into the skin from hydrogels prepared from xanthan gum. The enhanced drug transport into the skin is attributed to the lipid nature of the vesicles, which serve as carriers for the drug.

Treatment of human immunodeficiency virus (HIV) infections

Several antiretroviral nucleotide analogues have been developed for the treatment of patients suffering from the acquired immunodeficiency syndromes (AIDS). These include antisense oligonucleotide, which is a new antiviral agent that has shown potential therapeutic application against HIV-1. These antiviral agents are able to combat replication of the HIV by inhibiting reverse transcriptase and, thereby, viral DNA synthesis. However, these agents display a dose related toxicity. The effective dose can be reduced by encapsulation of such drugs in liposomes, thus reducing the incidence of toxicity [19]. The greater efficacy of the liposomal formulation relates to the preferential uptake of the liposomes into the virus compared with the host tissue.

Enhanced antimicrobial efficacy& safety [19]

Antimicrobial agents have been encapsulated in liposomes for two reasons. First, they protect the entrapped drug against enzymatic degradation. For instance, the penicillins and

cephalosporins are sensitive to the degradative action of β - lactamase, which is produced by certain microorganisms. Secondly, the lipid nature of the vesicles promotes enhanced cellular uptake of the antibiotics into the microorganisms, thus reducing the effective dose and the incidence of toxicity as exemplified by the liposomal formulation of amphotericin B.

Constraints in the commercialisation of liposomal preparation

Two major constraints are identifiable first, the lipids needed for their preparation are very scarce, should be of high purity and expensive. Secondly, liposomal preparations are inherently unstable and require special storage conditions (below $0^{o}C$) even when the products are freeze-dried [20]. As a result of this stability problem the dosage forms are limited to injection (freeze-dried) powders for reconstitution immediately before use. As a result of these constraints only a few products (e.g., liposomal amphotericin B) have actually been commercialised in spite of the large volume of research on liposomes.

Example of drugs in liposomal formulation

Table.1 Drug Application Commercial Name Composition of Liposomes

DRUG	APPLICATION	COMMERCIAL NAME	COMPOSITION OF LIPOSOMES
Amikacin	Bacterial Infection	MiKasome	HSPC/CH/DSPG
Adriamycin	Stomach Cancer	-	DPPC/CH
Ampicillin	Listeria monocytogenesis	-	CH/PC/PS
Annamycin	Breast, Cancer,Leukemia	Annamycin	Liposomes
Amphotericin B	Systemic FungalInfection	AmBisome	HSPC/CH/DSPG
All-*trans*retionic acid	Prostate Cancer, Leukemia	ATRAGEN	Liposomes
Muramyl dipeptide	Immunostimulator	-	DSPC/PS 1:1
Ciprofloxacin	*Pseudomonas aerogonisa*	-	DPPC
Clodronate	Macrophage suppresion	-	PC/CH
Cyclosporin	Immunosuppresor	-	PC/CH
Chloroquinine	Malaria	-	PC/PG/CH
Doxorubicin	Cancer	Doxil	HSPC/CH/PEG
Daunorubicin	Breast Cancer	Daunaxome	DSPC/CH
Gangiciclovir	HSV	-	Liposomes
Intralukin-2	Immunostimulant	-	DMPC
Mitoxantron	Colon Cancer	-	PC/CH
Methorexate	Cancer	-	DPPC/PI
Nystatin	Fungal Infection	Nyotran	Liposomes
Pentostam	Leishmanioses	-	Niosomes
Cisplastin	Mezotelioma	PLATAR	Liposomes
Lurotecon	Cancer	NX211	Liposomes
Prostaglandanin	Antiinflammatory	-	Liposomes
Ribavirin	Herpes Simplex	-	PC
Streptosotocin	Lymphocyte activator	-	DMPC/CH
Suramin	Trypanosomes	-	DPPC

CONCLUSION

This review showed that liposomes have been prepared from a variety of synthetic and naturally occurring phospholipids and generally contain cholesterol as membrane stabiliser.

Several methods of preparing liposomes were identified, which could influence the particle structure, degree of drug entrapment and leakage of the liposomes. It was also identified that there are improved pharmacokinetic properties with liposomal drugs compared to the free drugs. Furthermore, liposomes are tools for drug targeting in certain biomedical situations (e.g., cancer) and for reducing the incidence of dose related drug toxicity. Instability of the preparations is a problem, which is yet to be overcome before full commercialisation of the process can be realised.

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