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LIPOSOMES: AN OVERVIEW

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ABSTRACT:
Drug development technologies constituting innovations at the formulation end in the Pharmaceutical industry has received a lot of attention in past two decades. Drug delivery as an opportunity to extend product life cycles has indeed proved its place in the market with significant advantages of therapeutic gains as well as commercial success. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as liposomes, microspheres, nanoparticles, etc. which modulates the release and absorption characteristics of the drug. Liposomes are well known to alter the bio distribution of entrapped substances by protecting the enclosed material. They are widely used as vehicles to target the specific molecule to specific organ. During the last few decades liposomes have attracted great interest as ideal models for biological membranes as well as efficient carriers for drugs, diagnostics, vaccines, nutrients and other bioactive agents. Many techniques and methodologies have involved for the manufacture of liposomes, on small and large scales, since their introduction to the scientific community around 40 years ago. This article intends to provide an overview of the advantages and disadvantages of liposome preparation methods,their stability, bio distribution and their uses as drug delivery systems. The conventional method of preparing liposomes is basically for the multilamellar vesicles (MLVs). However, other methods are used to reduce the size of these MLVs to small unilamellar vesicles (SUVs) so as to increase their plasma lifetime and consequently increase the possibility of achieving greater tissue localisation. Some of these methods of size reduction are sonication and high pressure extrusion. Each of these methods has its own advantages and disadvantages. Large unilamellar vesicles (LUVs), on the other hand, are prepared mainly by detergent removal method and reverse phase extrusion technique. There are also improved pharmacokinetic properties with liposomal drugs compared to free drugs, though some formulation factors affect the release kinetics of the liposomal drugs. The review also shows that liposomes have a lot of biomedical applications and uses. They have been used in drug targeting, oral delivery of vaccines, insulin, peptides and some compounds, which are usually degraded in the gastrointestinal tract. It has also found application in topical therapy especially in the eye and lungs. Other areas of application are in cancer chemotherapy and treatment of human immunovirus (HIV) infection.
KEY WORDS: Carrier Systems, Lipid Vesicles, Phospholipids, Manufacturing Techniques, Applications and Limitation of Liposomes.

INTRODUCTION:

A tremendous amount of work has been done to formulate drugs in sustained and controlled release dosage forms for oral and parenteral administration[1]. To obtained an optimal drug action and targeting the drug to the particular sites in order to reduce the side effect and improve therapeutic efficacy by preventing undesired drug localization in healthy tissue sites and decreasing rapid degradation or elimination of drugs[2, 3, 4].

To pursue optimal drug action, functional molecules could be transported by a carrier to the site of action and released to perform their task, for which the carrier itself should be non toxic, biodegradable, and of suitable shape and size to accommodate wide variety of substances[5].

Among a variety of targeted drug carrier systems, Liposome science and technology is one of the fastest growing scientific fields contributing to areas such as drug delivery, cosmetics, structure and function of biological membranes and investigations of the origin of life to name a few. This is due to several advantageous characteristics of liposomes such as ability to incorporate not only water soluble but also lipid soluble agents, specific targeting to the required site in the body and versatility in terms of fluidity, size, charge and number of lamellae. While the use of liposomes as models for bio membranes is confined to the research laboratory, their successful application in the entrapment and delivery of bioactive agents will depend not only on a demonstration of the superiority of the liposome carrier for the intended purpose, but also upon technical and economic feasibility of the formulation[6].

Liposome was discovered about 40 years ago by Bangham and co-workers and was defined as microscopic spherical vesicles that form when phospholipids are hydrated or exposed to an aqueous environment[7]. Liposomes are microscopic vesicles composed of a bilayer of phospholipids or any similar amphipathic lipids. They can encapsulate and effectively deliver both hydrophilic and lipophilic substances[8, 9]. and may be used as a non-toxic Vehicle for insoluble drugs[10]. Weiner et al[11] Defined liposome as a microstructure consisting of one or more concentric spheres of lipid bilayer separated by water or aqueous buffer compartments. The typical characteristic of bilayer forming lipids is their amphiphilic nature: a polar head group covalently attached to one or two hydrophobic hydrocarbon tails. When these lipids like phosphatidyl choline, phosphatidyl ethanolamine or phosphatidyl glycerol, are exposed to an aqueous environment, interactions between themselves (hydrophilic interactions between polar head groups and van der wals interactions between hydrocarbon chains and hydrogen bonding with water molecules) lead to spontaneous formation of closed bilayers which is referred as a " LAMELLA[12,13].Liposomes can differ in size, ranging from the smallest vesicle (diameter 20nm) to liposomes that are visible under the light microscope with a diameter of 1µm or greater, equal to the dimensions of living cells[14].Liposome can carry drugs in one or three potential compartments (water soluble agents in the central aqueous core, lipid soluble agents in the membrane, peptide and small proteins at the lipid aqueous interface). They are classified structurally into multilamellar vesicles (MLVs) and unilamellar vesicles (ULVs)[15]. ULVs have a single phospholipids bilayer membrane and a diameter of 0.05– 0.25 µm. These liposomes (i.e., ULVs) can be further classified into large unilamellar vesicles (LUVs) with a diameter of 0.05– 0.25µm and small unilamellar vesicles (SUVs) with a diameter of 0.05–0.10 µm.

Liposomes are designed in such a way that the solute can be entrapped in the aqueous compartment (polar solute) or embedded in the lipid bilayers (lipohilic or amphiphilic solute). Since ULVs contain a large central aqueous

compartment, they are ideally suited for the encapsulation of water soluble agents[16]. MLVs are composed of concentric phospholipids bilayer membranes in an onion skin arrangement and have a diameter of 1–5 μm. ULVs contain a small aqueous compartment (<10%), which means that they preferentially entrap lipid soluble drugs[16]. This observation is important to the design of liposomes for cancer therapy since a number of active cytotoxic drugs are highly lipid soluble[17].

Liposomes have become a valuable experimental and commercially important drug delivery system, owing to their biodegradability, biocompatibility, low toxicity and ability to entrap lipophilic and hydrophilic drugs[18-23]. Present applications of the liposomes are in the immunology, dermatology, vaccine adjuvant, eye disorders, brain targeting, infective disease and in tumour therapy[24-26].

MATRIALS USED FOR PREPARATION OF LIPOSOMES[27]:

Liposomes can be prepared from a variety of lipids and mixtures. Phospholipids are most often used especially phosphatidylcholines which are amphopathic molecules in which a glycerol bridge links a pair of hydrophobic acylhydrocarbon chains with a hydrophilic polar head group. Phosphatidylcholines contrast markedly with other amphipathic molecules(detergents, lysolecithins) in that bilayer sheets are formed in Preference to micellar structures because the double fatty acid chains give the molecule an overall tubular shape, more suitable for aggregation in planar sheets than in other aggregate structure.

Phosphatidyl cholines : also known as lecithin, can be derived from both natural and synthetic sources. They are readily extracted from egg yolk and soya bean but less readily from bovine heart and spinal cord. They are often used as the principal phospholipids in liposomes for a wide range of application because of their low cost relative to other Phospholipids, their neutral charge, and their chemical inertness. Lecithin from plant sources has a high level of polyunsaturation in the fatty acyl chains, while that from mammalian sources contains a higher proportion of fully saturated chains.

Phospholipids: natural phospholipids have two hydrocarbon chains are linked to a phosphate-containing polar head group. In phosphoglycerides the linkage of fatty acid to head group is via a bridge region consisting of the three carbon glycerol. In sphingolipids, the lipid sphingosine forms one of the hydrocarbon chains and it links directly to the phosphate. Phospholipids can possess fatty acid of different chain length and unsaturation and may have different hydrophilic species linked to phosphate, according to which individual members of the phospholipids category are classified.

Phosphatidyl ethanolamine (PE): has a similar head group as Phosphatidylcholines and the presence of hydrogen's directly attached to the nitrogen of ethanolamine permits interactions of adjacent molecules in the membrane by hydrogen bonding. At low or neutral pH, the amino group is protonated, giving a neutral molecule, which prefers to form hexagonal II phase inverted micelles to lamellar structures when above the main phase transition temperature.

Phosphatidyl glycerol (PG): possess a permanent negative charge over the normal physiological pH range. In addition, it is directly isolated from natural sources & readily prepared semi-synthetically from other lipids by the action of phospholipase D in the presence of glycerol.

Phosphatidyl serine (PS): is linked to the phosphate via its hydroxyl group, leaving the carboxyl and amino functions both free and ionized to from a neutral zwitter ion. The net charge of the PS head group is therefore negative as a result of the charge on the phosphate. Membranes containing PS show a marked sensitivity to calcium, which interacts directly with the carboxyl functions on the head groups, causing PS molecules to aggregate within the membrane resulting in a condensed phase separate from that of the bulk lipids. Together with this phase separation goes the appearance of packing irregularities at phase boundaries. Calcium also causes bridging interactions between PS on membranes of different liposomes, so that aggregation of these liposomes, in which packing defects have been introduced, often results in fusion. However, it has been reported that the presence of PS in membranes helps to stabilize them during freeze-drying in the presence of sugars.

Phosphatidic acid (PA): Absence of any substitution on the phosphate in PA confers a very strong negative charge to the molecule. Dispersions of PA alone in water have a pH of between 2 and 3, and rapid neutralization with acid can cause membrane reorganization, under the influence of electrostatic effect to produce unilamellar vesicles.

Sphingo myelin(SM): is found to varying extents in the erythrocyte plasma membranes of a number of mammalian species and completely replaces PC in sheep red cells. It is also readily extracted from nervous tissue. It is a neutral molecule with the same phosphocholine head group as PC. SMs have hydrocarbon chains often markedly different in length and with a degree of unsaturation giving rise to transitition between 20˚C and 40˚C. Membrane packing is tighter than for PC by virtue of the extra hydrogen bonding made possible in the bridge region by the presence of the amide hydrogen, which participates in interaction between adjacent sphingomyelin molecules, and probably also with cholesterol.

Lyso-phospholipids: lipids described above other then lyso-phospholipids can lose a fatty acid chain by either chemical or enzymatic hydrolysis to give single chain amphiphiles. While they do not from membranes themselves. They are often present in membranes as impurities either of the starting components or as a result of degradation during storage. In high concentrations lysophospholipids can disrupt membranes and they can be highly toxic for cells and whole organisms. Membrane disruption with LPC only occurs when there is an imbalance in chains in the membrane relative to the head groups. The action of phospholipase A converting PC to LPC and fatty acid does lead to perturbations until the fatty acid has been removed from the membrane (e.g. by incubating with albumin) where upon increase in permeability.

CLASSIFICATION OF LIPOSOMES:

On The Basis Of Composition: liposomes are composed of natural and/or synthetic lipids (phospho and sphingo-lipids) and may also contain other bilayer constituents such as cholesterol and hydrophilic polymer conjugated lipids. The net physicochemical properties of the lipids composing the liposomes such as membrane fluidity, charge density, stearic hindrance and permeability determine liposome's interactions with blood components and other tissues after systemic administration. The nature and extent of liposome-cell interaction in turn determines the mode of intracellular delivery of drugs. Thus, the predominant mechanism behind intracellular delivery of drugs by liposomes may mainly depend on their composition. Liposomes can be classified in terms of composition and mechanism of intracellular delivery

into five types as: (1).Conventional liposomes (CL) (2). pH-sensitive liposomes (3).Cationic liposomes (4).Immunoliposomes (5). Long-circulating liposomes (LCL)[28].

On The Basis Of Size: the liposome size can range from very small to large vesicles which are shown in **figure 1**. Furthermore, liposomes may have single or multiple bilayer membranes. The vesicle size is a critical parameter in determining circulation half life of liposomes and both size and number of bilayers influence the extent of drug encapsulation in the liposomes. On the basis of their size and number of bilayers, liposomes can also be classified into one of three categories: (1). Multilamellar vesicles (MLV) (2). Large unilamellar vesicles (LUV) (3). Small unilamellar vesicles (SUV)[28].

METHODS FOR PREPARATION OF LIPOSOMES:

Multilamellar Liposomes (MLV)

1).Lipid Hydration Method (Hand shake vesicles): is the most widely used method for the preparation of MLV. This method involves drying a solution of lipids so that a thin film is formed at the bottom of round bottom flask and then hydrating the film by adding aqueous buffer and vortexing the dispersion for some time. The hydration step is done at a temperature above the gel-liquid crystalline transition temperature Tc of the lipid or above the Tc of the highest melting component in the lipid mixture. The compounds to be encapsulated are added either to aqueous buffer or to organic solvent containing lipids depending upon their solubility's. MLV are simple to prepare by this method and a variety of substances can be encapsulated in these liposomes. The drawbacks of the method are low internal volume, low encapsulation efficiency and the size distribution is heterogeneous[29].

2). Solvent Spherule Method: This process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath[30].

Small Unilamellar Liposomes (SUV)

1).Sonication Method: Here MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere. The main drawbacks of this method are very low internal volume encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV along with SUV [31].

2).French Pressure Cell Method: This method involves the extrusion of MLV at 20,000 psi at 4°C through a small orifice. This method has several advantages over sonication method. This method is simple rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of this method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum)[32].

Large Unilamellar Liposomes (LUV): They have high internal volume/encapsulation efficiency and are now a day's being used for the Encapsulation of drugs and macromolecules.

1). Solvent Injection Methods:-

a. Ether Infusion Method: A solution of lipids dissolved in diethyl ether or ether/methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at 55-65°C or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The main drawbacks of the method are that he population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature[33, 34].

b.Ethanol Injection Method: A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all ethanol because it forms azeotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol[35].

2).Detergent Removal Methods: The detergents at their critical micelles concentrations have been used to solubilise lipids. As the detergent is removed the micelles become progressively richer in phospholipid and finally combine to form LUVs. The detergents were removed by dialysis[36,37,38].The advantages of detergent dialysis method are excellent reproducibility and production of liposome populations which are homogenous in size.The main drawback of the method is the retention of traces of detergent(s) within the liposomes. A commercial device called LIPOPREP (Diachema AG,Switzerland) which is a version of dialysis system is available for the removal of detergents. Other techniques have been used for the removal of detergents: (a) By using Gel Chromatography involving a column of Sephadex G-25[39]. (b) By adsorption or binding of Triton X-100 (a detergent) to Bio-Beads SM-2[40]. (c) By binding of octyl glucoside (a detergent) to Amberlite XAD-2 beads[41].

3).Reserves Phase Evaporation Method: First water in oil emulsion is formed by brief sonication of a two phase system containing phospholipids in organic solvent (diethyl ether or isopropyl ether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl.The method has been used to encapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or DNA strands[42], and get a heterogeneous sized dispersion of vesicles by this method.

4).Calcium-Induced Fusion Method: This method is used to prepare LUV from acidic phospholipids. The procedure is based on the observation that calcium addition to SUV induces fusion and results in the formation of multilamellar structures in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs[43]. The main advantage of this method is that macromolecules can be encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, although of a heterogeneous size range. The chief disadvantage of this method is that LUVs can only be obtained from acidic phospholipids.

5).Microfluldization Method: The microfluidization / microemulsification / homogenization for the large scale manufacture of liposomes. The reduction in the size range

can be achieved by recycling of the sample. The process is reproducible and yields liposomes with good aqueous phase encapsulation[44]. Riaz and Weiner (1995)[45] prepared liposomes consisting of egg yolk, cholesterol and brain phosphatidyl serine disodium salt (57:33:10) by this method. First MLV were prepared by these were passed through a Micro fluidizer (Microlluidics Corporation, Newton, MA, USA) at 40 psi inlet air pressure. The size range was 150-160 nm after 25 recycles. In the Micro fluidizer, the interaction of fluid streams takes place at high velocities (pressures) in a precisely defined micro channels which are present in an interaction chamber. In the chamber pressure reaches up to 10,000 psi this can be cause partial degradation of lipids.

(9).Freeze-Thaw Method: SUVs are rapidly frozen and followed by slow thawing. The brief sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is due to the fusion of SUV during the processes of freezing and or thawing. This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipids concentration. The encapsulation efficiencies from 20 to 30% were obtained.[45-48].

Giant Liposomes: The procedure for the formation of giant liposomes involves the dialysis of a methanol solution of phosphatidylcholine in the presence of methylglucoside detergent against an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm[49].

Multivesicular Liposomes: The formation of multivesicular liposomes involves the water in oil emulsion was converted to organic solvent spherules by the addition of the emulsion to across solution. The evaporation of organic solvent resulted in the formation of multivesicular vesicles. The diameter of liposomes ranges from 5.6 to 29 mm. The materials which can be encapsulated include glucose, EDTA, human DNA. These liposomes have very high encapsulation efficiency (up to 89%)[50].

Assymetric Liposomes: It has been shown that the phospholipid distribution in natural membranes is asymmetric. For example phosphatidylcholine and sphingomyelin concentrate at the outer half of lipid bilayer whereas phosphatidyl ethanolamine, phosphatidyl linositol and phosphatidyl serine are mainly localized in the inner half of bilayer.[51] Due to this attempts have been made to prepare LUVs in which phospholipids distribution in both half's of bilayer is different. It appears that as model membranes the asymmetric liposomes are nearer to natural membranes than the conventional unilamellar liposomes. In the latter the phospholipids distribution is symmetrical in bilayer. **Cestaro et al., 1982**[52] described a procedure for the preparation of asymmetric liposomes which contain cerebroside sulphate only at the outer leaflet of phospholipids bilayer. Cerebroside sulphate was adsorbed on to a filter paper (cellulose) support and then the support was incubated with small or large fused unilamellar liposomes. After six hours sulphated contents reached about 6 mole percentage of the total quantity of phospholipids, corresponding to about 10 mole % of phospholipids present in the outer layer. The sulphated could not be removed by washing with 1M NaCl or 1M urea.

Other Methods:

a. Lyophilization: Freeze-drying (lyophilization) involves the removal of water from products in the frozen state at extremely low pressures. The process is generally used to dry products are thermo labile and would be destroyed by heat-drying. The technique has a great potential as a method to solve long term stability problems with respect to liposomal stability. It is exposed that leakage of entrapped materials may take place during the process of freeze- drying and on reconstitution. Recently, it was shown that liposomes when freeze-dried in the presence of adequate amounts of trehalose (a carbohydrate commonly found at high concentrations in organism) retained as much as 100% of their original contents. It shows that trehalose is an excellent cryoprotectant (freeze-protectant) for liposomes[53].

b. **Heating Method:** This method involves the hydration of the liposome components in an aqueous medium followed by the heating of these components in presence of glycerol (3% v/v) up to 120°C . Glycerol is a water-soluble and physiologically acceptable chemical with the ability to increase the stability of lipid vesicles and does not need to be removed from the final liposomal product. Since heating is the main step in this methodology it is termed Heating Method and the resultant liposomes are referred to as HM-liposomes. It was confirmed by TLC that no degradation of the lipids occurred at the above mentioned temperatures employment of heat. In fact, it abolishes the need to carry out any further sterilisation procedure hence reducing the time and cost of liposome production by the heating method[54-56].

Application of glycerol in the preparation of the HM-liposomes has the following advantages:

1. Glycerol is a bioacceptable, non-toxic agent already in use in many pharmaceutical products and can serve as an isotonising agent in the liposomal preparations.
2. Unlike the volatile organic solvents employed in the manufacture of conventional liposomes, there is no need for the removal of glycerol from the final preparation
3. It serves as dispersant and prevents coagulation or sedimentation of the vesicles thereby enhancing the stability of the liposome preparations;
4. It also improves the stability of the liposome preparations against freezing, thawing[57].

c. High-Pressure Extrusion Method: This is another method for converting MLV to SUV suspensions. By this method, suspensions of MLVs prepared by the conventional method are repeatedly passed through filters polycarbonate membranes with very small pore diameter (0.8–1.0 μm) under high pressure up to 250psi. By choosing filters with appropriate pore sizes, liposomes of desirable diameters can be produced. The mechanism of action of the high pressure extrusion method appears to be much like peeling an onion. As the MLVs are forced through the small pores, successive layers are peeled off until only one remains. Besides reducing the liposome size, the extrusion method produces liposomes of homogeneous size distributions. A variety of different lipids can be used to form stable liposomes by this method[58-62].

FACTORS AFFECTING ON LIPOSOMES FORMULATION:

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/ or 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.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/or incorporation of charged lipids, e.g., phosphatidyl serine into bilayers are measures for increasing the aqueous volume in liposomes[64-65]. 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, 2.3–11.6% 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 stabilising 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[63-65].

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. 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[66].

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/lipophobic 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 minimise leakages, liposomes are stored in the form of freeze-dried powders[67-70].

APPLICATION OF LIPOSOMES:-

Protection against enzymatic degradation of drugs: Liposomes are used to protect the entrapped drug against enzymatic degradation whilst in circulation[7]. The basis is that the lipids used in their formulation are not susceptible to enzymatic degradation so 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 or by dissolution of the shell or degradation of the shell by lysosomal enzymes. Thus, β-lactamase sensitive antibiotics, e.g., penicillin and cephalosporin have been encapsulated due to this reason to protect against the β- lactamase enzyme. Liposome also offer protection for its encapsulated drugs in the environment of the gastrointestinal tract and facilitate the gastrointestinal transport of a variety of compounds. Liposome is therefore candidates to be explored for oral delivery of insulin and proteins for use as vaccines, which are otherwise orally degradable. Liposome use as carriers of vaccine agents because they are biodegradable and non-toxic. Twenty five years after the discovery of the immunological adjuvant properties of liposome, they are now considered the major candidate as the base for oral vaccine against hepatitis A, which is being licensed for use in humans[71-75].

1. Drug targeting: The need for "drug targeting" arises from a problem situation whereby a drug administered 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 intracellular 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 ligands recognise specific receptor sites and thus cause the lipid vesicles to concentrate at such target sites. By this approach the otherwise preferential distribution of liposome into the reticuloendeothelial system RES (liver, spleen and bone marrow) is minimised. A ligands 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[8, 76-78].

2. 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 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[79-82].

4. 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 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. The greater efficacy of the liposomal formulation relates to the preferential uptake of the liposomes into the virus compared with the host tissue[83-84].

5. Enhanced antimicrobial efficacy/ safety: 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[85].

6. Cationic liposomes for gene delivery: Among various synthetic carriers currently in use in gene therapy, cationic liposomes are the most suitable transfecting vectors. Gene encapsulation in liposomal vesicles allows condensation of DNA plasmid into a highly organized structure and protects DNA against degradation during storage and in the systemic circulation of the gene encoding a therapeutic protein. Moreover, structural organization of the gene-delivery system must bypass the cell membrane and facilitate endosomal escape avoiding DNA degradation in the lysosomal compartment. Numerous cationic lipids have been tested in the formulation of liposomes for gene delivery. Transfection efficiency is strongly affected by the presence of three components in the structure of lipids: a positively charged head-group that interacts with negatively charged DNA, a linker group (which determines the lipid's chemical stability and biodegradability), and a hydrophobic region to anchor the cationic lipid into the bilayer[86].

7. Liposomes for diagnostic imaging: Actively or passively targeted liposomes can be used as carriers for contrast agents to increase the signal difference between areas of interest and background and to specifically localize the contrast moieties in the target tissues or organs. The versatility of liposomal vesicles to carry different types of compound in the bilayer or in the aqueous compartment makes them suitable for all contrast procedures including gamma-scintigraphy, magnetic resonance imaging (MRI), computed tomography imaging (CTI) and sonography. Using liposomes in diagnostic imaging leads to several advantages owing to their capability to incorporate multiple contrast moieties to specifically deliver the agent to the target area and to enhance the contrasting signal. In order to incorporate diagnostic agents (111In, 99Tc, Mn, Gd, etc) in liposomes, metals can be complexed with a soluble chelating agent (such as DTPA) that will be encapsulated in the aqueous core of the vesicles. Alternatively, the chelating compound complexing with the metal can be derivatized with a hydrophobic group for insertion in the lipid bilayer. So, in these cases metals are situated on the liposomal surface, directly exposed to the aqueous environment, thus enhancing the contrast proprieties[87].

8. Liposomes for vaccines: Genetic vaccination-encoding antigens from bacteria, virus and cancer have shown promise in protecting humoral and cellular immunity. The success of liposomes-based vaccines has been demonstrated in clinical trials and further human trials are also in progress. Liposomes are of interest as carriers of antigens especially because they act as effective adjuvants for the immune system response without causing granulomas at the injection site and producing no hypersensitivity reactions.Liposome formulations would also protect their DNA content from deoxyribonuclease attack. Moreover, their transfection efficiency could be improved by modulating surface charge, size, and lipid composition of the vesicle and entrapping additional adjuvant or immunostimulator compounds in the antigen formulation. Several strategies have been followed to target liposomes to cell receptors, such as antibodies (or Fc-γ) or branched chain mannose moieties. Cationic or pHsensitive liposomes that are able to release their contents into the cytoplasm following endocytosis have also been developed[88].

9. Other pharmaceutical uses of liposomes: Active research is in progress in the area of liposomes for use as vesicular containers, in particular for haemoglobin as blood substitute. Liposome-encapsulated haemoglobin (LEH) is being developed as an oxygen therapeutic. The spatial isolation of haemoglobin by a lipid bilayer potentially minimizes the cardiovascular/hemodynamic effects associated with other modified forms of hemoglobin; moreover, it is possible to co-encapsulate reductants, antioxidative enzyme systems, and oxygen-affinity modifiers with haemoglobin so as to artificially recreate the red blood cell environment. The circulation half-life is 65 hours for this PEG-LEH formulation; the results demonstrate that LEH circulates for a prolonged time after administration and that the animals tolerate at least 25% of blood exchange without any distress[89-90].

LIMITATION OF LIPOSOMES:

As described above, liposomes have a great potential in the area of drug delivery. However, liposome-based drug formulations have not entered the market in great numbers so far. Some of the problems limiting the manufacture and development of liposomes have been stability issues, batch to batch reproducibility, sterilization method, low drug entrapment, particle size control and production of large batch sizes and short circulation half-life of vesicles. Some of these issues such as short half-life have been resolved resulting in increased numbers of clinical trials. Some of the remaining problems are following:-

1. Stability: One of the major problems limiting the wide spread use of liposomes is stability--both physical and chemical. Depending on their composition the final liposome formulations may have short shelf-lives partly due to chemical and physical instability. Chemical instability may be caused by hydrolysis of ester bond and/or oxidation of unsaturated acyl chains of lipids. Physical instability may be caused by drug leakage from the vesicles and/or aggregation or fusion of vesicles to form larger particles. Both of these processes (drug leakage and change in liposome size) influence the in vivo performance of the drug formulation, and therefore may affect the therapeutic index of the drug. For instance, large liposomes may be rapidly cleared by RES leading to sub therapeutic plasma concentrations of the drug and reduced AUCs (area under the plasma concentration-time curve). Physical instability may also occur due to partitioning out of a hydrophobic drug from the bilayer into the solvent on standing (or long term storage). Some of the stability problems may be overcome by lyophilization in which the final liposome product is freeze-dried with a cryoprotectant (mostly a sugar like Trehalose) and is reconstituted with vehicle immediately prior to administration. Lyophilization increases the shelf-life of the finished product by preserving it in a relatively more stable dry state. Some liposome products on market or in clinical trials are provided as a lyophilized powder. For example, AmBisome TM[91-92].

2. Sterilization: Identification of a suitable method for sterilization of liposome formulations is a major challenge because phospholipids are thermo labile and sensitive to sterilization procedures involving the use of heat, radiation and/or chemical sterilizing agents. The method available for sterilization of liposome formulations after manufacture is filtration through sterile 0.22 μm membranes. However, filtration is not suitable for large vesicles (>0.2 μm) and also is not able to remove viruses. Sterilization by other approaches such as $^{\gamma}$- irradiation and exposure to chemical sterilizing agents are not recommended because they can

cause degradation of liposome components and may leave toxic contaminants. under certain conditions, liposomes with thermostable , lipophilic drugs could be sterilized by autoclaving without substantial loss of contents and/or degradation of phospholipids[93-94].

3. Encapsulation efficiency: Liposome formulation of a drug could only be developed if the encapsulation efficiency is such that therapeutic doses could be delivered in a reasonable amount of lipid. Since lipids in high doses may be toxic and also cause non-linear (saturable) pharmacokinetics of liposomal drug formulation. Some new approaches that provide high encapsulation efficiencies for hydrophilic drugs have been developed. For instance, active loading of the amphipathic weak acidic or basic drugs in empty liposomes can be used to increase the encapsulation efficiency. However, active loading is not suitable for hydrophobic drugs such as paclitaxel for which encapsulation efficiency is < 3 mole% mainly due to the low affinity of drug for the lipid bilayers[91,95-97].

4. Lysosomal degradation: One of the major limitations of active targeting using ligand-directed immunoliposomes has been their rapid clearance due to non-specific uptake by the cells of RES. The ligand (antibodies) conjugated with liposomes may increase the liposome size and reduce extravasation and thus could limit targeting to intravascular targets.Moreover, immunoliposome enter the cells by endocytosis and if liposome contents are not released from the endosome they would ultimately be degraded in the lysosomes[98].

5. Gene therapy: A number of technical problems have to be overcome before cationic liposome-mediated transfection can be fully exploited. For instance, liposomes are significantly less efficient than viral vectors in their transfection ability. Furthermore, the DNA-lipid complexes are not stable in terms of particle size for long periods of time. In addition, there is lack of in vivo targeting after systemic administration and the toxicity of cationic lipids limits the administered dose of the DNA lipid complex. Plasmid-liposomes complexes may be more suited to delivery of genetic material by local administration[99-100].

6. Active Targeting: Once the liposome has reached the target cell, the efficacy is determined not only by the amount of drug associated with the cell, but also by the amount of drug reaching the 'target molecule' inside the cells. Immunoliposomes may deliver the drug to the cells selectively but the pharmacological activity depends on the ability of intact drug to diffuse into cytoplasm from the endosomes in sufficient amounts[101].

CONCLUSION:

The development of liposomes as carriers for therapeutic molecules is an ever-growing research area. The possibility of modulating the technological characteristics of the vesicles makes them highly versatile both as carriers of several types of drugs (from conventional chemotherapeutics to proteins and peptides) and in therapeutic applications (from cancer therapy to vaccination). In recent years, several important formulations for the treatment of different diseases have been developed. Liposomes allowed a significant vesicular carrier system for therapeutic effectiveness in terms of duration of action and decrease in dose frequency and delivering drugs at a higher efficacy and lower toxicity. They do, however have their limitations and as far as drug delivery goes there seems to be an emphasis on the use of sterically stabilized liposomes.

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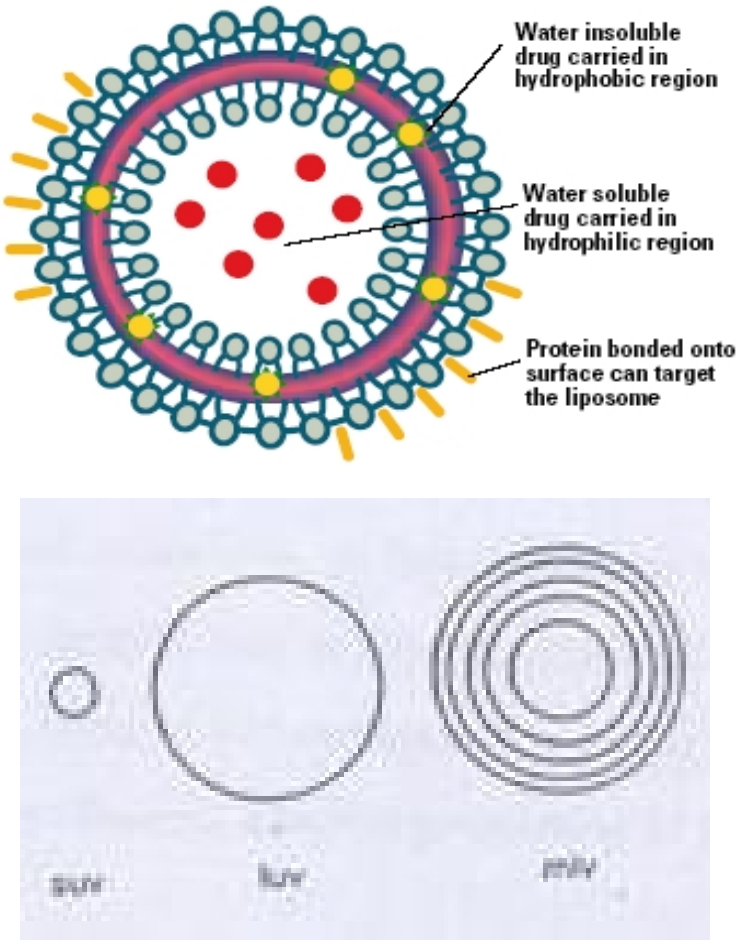

Figure: 1. Structure of liposomes