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"Liposomes - An Updated overview"

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Abstract: Due to their great biocompatibility, biodegradability, and minimal immunogenicity, liposomes are now the most widely employed nanocarriers for a variety of hydrophobic and hydrophilic compounds that may be biologically active. Additionally, liposomes demonstrated improved drug solubility and regulated distribution. They also showed the ability to modify the surface of drugs for targeted, extended, and sustained release. Liposomes can be thought of as having developed from traditional, long-circulating, targeted, and immunological liposomes to stimuli-responsive and actively targeted liposomes based on their composition. More liposomes have advanced stages in clinical trials, and many liposomal-based drug delivery systems are currently clinically licensed to treat several disorders, including cancer, fungal infections, and viral infections. This review discusses the composition, preparation procedures, and clinical uses of liposomes.

1. Introduction:

When phospholipids are dispersed in water, they spontaneously form a closed structure with an internal aqueous environment bounded by phosphor lipid bi-layer membranes, this vesicular system is called a liposome. Liposomes are the small vesicle of spherical shape that can be produced from cholesterols, non-toxic surfactants, sphingolipids, glycolipids, long-chain fatty acids, and even membrane proteins.[1]

1.1 Structure of liposome:

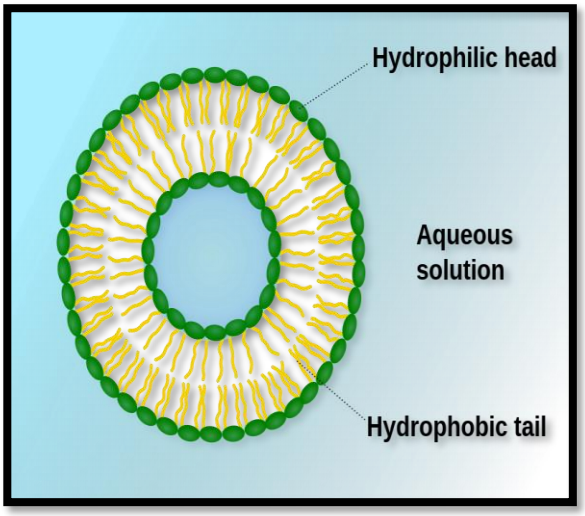

Figure 1: Scheme of a liposome formed by phospholipids in an aqueous solution

Drug delivery systems (DDSs) offer the potential to enhance the therapeutic index of drugs by increasing the drug concentration, and the residence time in target cells and minimizing the side effects.[2] The interaction of liposomes with proteins, particularly those proteins found in blood serum, becomes a matter of considerable concern. The interaction with serum proteins can affect the stability of the liposome and also its ability to interact with the cells of body.[3] A general overview of liposomes as analytical tools is given by Edwards *et al.*. In this report, techniques and methods are described that can be used to quantitatively describe liposomes. This provides a means of comparing different batches of liposomes and generates data that assist in the understanding of liposomes and their use in a variety of different application areas.[4]

Described first in the 1960s by Bangham1 and understood as a potential drug delivery system in the early 1970s, the liposome has since become integral to research and clinical applications in the field of nanomedicine. Five decades of research in the field of liposome research have shown their prospective benefits in the medical and cosmetic as well as the food industry.[5] The use of liposomes in cosmetics is generally linked to the efficient delivery of moisturizing ingredients (including water) to the skin. A recent presentation at the Controlled Release Society Workshop in Geneva suggested that liposomes can be used to deliver oxygen to the skin which will retard skin aging. The applications for liposomes have increased to include the delivery of both fat-soluble and aqueous-soluble food ingredients.[6]

resonance and electron spin resonance), the binding of local anesthetic molecules to membranes, and the actions of the anesthetics on these membranes, the freezing of living cells, mechanisms of inflammation in gout, the reconstitution of membrane-bound ion-transport systems, and interactions of antibiotics with membranes.[8] Due to recent developments in liposome technology, more effective strategies are now available for controlling the stability and reactivity of liposomes after systemic administration (Lasic and Papahadjopoulos, 1995).[9]

1.2 Types of Liposomes:[10]

There are three types of liposomes:

1. MLV (Multilamellar vesicles)

2. SUV (Small unilamellar vesicles)

3. LUV (Large unilamellar vesicles)

1.3 Properties of Liposomes:[10]

The system is composed of structures of bimolecular sheets intercalated by aqueous space.

• They are permeable to water.

• They are osmotically sensitive.

• Positively charged membranes are impermeable to cations and negatively charged ones are relatively permeable to anions.

Antioxidant liposomes hold great promise in the treatment of many diseases in which oxidative stress plays a prominent role.[11] Since liposomes are used as carriers for a very wide variety of drugs and other compounds we have not included toxicity tests for specific drugs but provide details of simple in vitro screening tests and a general outline of the tests required for drug registration.[12] Skin is the largest, easily accessible organ for local and systemic drug administration. But the skin behaves as a passive barrier to the penetrant molecule. Cosmeceuticals refer to the combination of cosmetics and pharmaceuticals. Cosmeceuticals are cosmetic products with biologically active ingredients purporting to have medical or drug-like benefits.

Some cosmeceuticals can act effectively when reaching their target sites in deeper layers of the skin. The main barrier is located in the outermost layer of the skin, the stratum corneum, which contains flattened dead epidermal cells (corneocytes) embedded in hydrophobic lipid domains. The stratum corneum provides the greatest resistance to penetration, and it is the rate-limiting step in percutaneous absorption.[13]

An important factor in liposome-cell interactions is the fluidity of the liposome bilayer. Each phospholipid species is characterized by a gel-liquid crystalline phase transition temperature, below which its fatty acyl chains are in a quasi-crystal.

Line array and above which the chains are in a more fluid state. Decreased bilayer thickness and increased area per molecule also accompany chain melting. These changes have been studied extensively by a variety of physical techniques, including X-ray diffraction, nuclear magnetic resonance, electron spin resonance, differential scanning calorimetry, and fluorescence depolarization. In general, the transition temperature is lowered by decreased chain length, by unsaturation of the acyl chains, by bulky side groups (e.g. cyclopropane rings), and by branching of the acyl chains. The polar head group also influences the transition, but to a lesser extent than the acyl chains.[14] Compartmentalization is one of the key steps in the evolution of cellular structures and, so far, only a few at- tempts have been made to model this kind of "compart- mentalized chemistry" using liposomes.

The present work shows that even such complex reactions as the ribosomal synthesis of polypeptides can be carried out in liposomes. A method is described for incorporating into 1-palmitoyl-2-oleoyl-sn-3-phosphocholine (POPC) liposomes the ribosomal complex together with the other components necessary for protein expression. Synthesis of poly(Phe) in the liposomes is monitored by trichloroacetic acid of the ^{14} C-labelled products. Control experiments carried out in the absence of one of the ribosomal subunits show by contrast no significant polypeptide expression. This methodology opens up the possibility of using liposomes as minimal cell bioreactors with grow- ing degree of synthetic complexity, which may be relevant for the field of origin of life as well as for biotechnological applications.[15]

A significant advantage of liposomes is that they can incorporate and release two materials with different solubilities simultaneously. One example of this is the incorporation of two antioxidant agents namely alpha-tocopherol (a lipid-soluble molecule) and glutathione (a water-soluble molecule) in the same lipid vesicle.[16] Since lysosomal storage diseases are caused by a genetic deficiency of specific acid hydrolases in lysosomes, the reversal of such enzyme deficiencies has been approached by employing direct enzyme replacement. Because it is the affected system, the lysosomal apparatus of cells, that normally takes up extracellular macromolecules and particles by endocytosis, attempts have been made to mobilize the stored GM,-ganglioside present in Tay-Sachs disease by direct administration of purified enzyme. Un-fortunately, the injected enzyme disappears rapidly from the circulation and most of it becomes localized in the liver rather than at other affected sites, such as the central nervous system.[17]

In the late nineteenth century, German bacteriologist Paul Ehrlich, used the term "magic bullet," which means chemical carriers that have the property of selectivity in killing abnormal cells without any effect on the normal ones. To improve this specificity through drug delivery systems, there are a variety of different approaches, which are based on several physical and bio-chemical principles.[18] Liposomes are the most common and well-investigated nanocarriers for targeted drug delivery. They have improved therapies for a range of biomedical applications by stabilizing therapeutic compounds, overcoming obstacles to cellular and tissue uptake, and improving the biodistribution of compounds to target sites *in vivo*.[19]

Liposomes are microscopic and submicroscopic vesicles with sizes ranging from 10 nm to 20 nm. They are composed of one or several lipid bilayers enclosing aqueous compartments. When phospholipids (PL) are hydrated, they spontaneously form lipid spheres enclosing the aqueous medium and the solute.[20] At present,

these include antifungal and anti-cancer preparations that compare favorably to existing treatments, but a recent renaissance in liposome research is promising many more products to come.[21] The types of materials which can be entrapped in liposomes are quite variable and, as will be discussed in detail in later sections, in addition to ions and proteins, includes several drugs.[22] In the lotions aisle of the local pharmacy, you may see several skin products that advertise the presence of liposomes microscopic spheres that help reduce the signs of as one manufacturer aging," as one manufacturer promises.

Liposomes also have a variety of less frivolous applications, ranging from basic research on the properties of biological membranes to the delivery of therapeutic drugs.[23] The unique versatility of lipid vesicles concerning composition, size variety, and capacity for embedding and encapsulating materials has led to applications in chemical and biochemical analytics and even to industrial-scale applications in drug delivery, cosmetics, food technology, and proteomics. Liposomes are commonly utilized as precursors in the fabrication of suspended bilayers, and recently, nanotubes-conjugated liposome networks have emerged as novel biomimetic chemical reactor systems with capabilities for single-molecule analysis.[24]

An oral formulation, and subsequent ingestion, is the most common route of obtaining proper nutrition. Food and other biologically active ingredients are digested and adsorbed in the gastrointestinal (GI) tract, facilitated by this route of entry. Although our existence demonstrates that this is an effective process, we must be aware that many molecules are poorly adsorbed. Many oral drugs, vitamins, and herbs, in which very low levels, sometimes even below 10%, are being absorbed into the bloodstream. The mechanism by which gastrointestinal absorption takes place is either a passive or an active transport process. In the former case, the drug crosses the intestinal lumen by passive diffusion across the enteric cells and distribution between the aqueous and membrane phase, while the active transport mechanism is facilitated by cell membranes which contain specific receptor proteins that take up, in an ATP-driven process, interacting molecules such as amino acids and cholesterol.[25]

Treatment of human disorders by the direct administration of drugs can be hampered by immunological reactions, development of drug resistance, uptake of drugs by non-diseased tissues often leading to serious side effects, or even by the inability of some drugs to penetrate target cells. Experiments with liposomes-entrapped and non-entrapped drugs injected into rats revealed that entrapment directed to a considerable extent the rate of drug elimination from the plasma and drug tissue distribution.[26] Recently, accumulating information on the three-dimensional structure of drugs and on quantitative structure activity relationships has contributed to better drug design and optimization of the interaction of at least some drugs with their respective molecular targets. However, it is more than likely that, in spite of progress in this area, many of the aforementioned obstacles will remain, particularly those encountered within the biological milieu interposed between the site of drug administration and the target. On the other hand, developments in such unrelated areas as hybridoma and recombinant DNA technology, the chemistry of polymers and colloids and the under- standing of ligand receptor interactions and ensuing intracellular events have now formed a realistic basis for the pursuit of an alternative approach to conferring selectivity on drugs, namely targeted drug delivery.[27] Liposomes were first produced by Bangham while investigating the role of phospholipids in blood clotting. Liposomes are microscopic, spherical vesicles that form when hydrated phospholipids arrange themselves in circular sheets with consistent head-tail orientation. These sheets join others to form a bilayer membrane that encloses some of the water and water-soluble material in a phospholipid sphere.[28]

Due to their structure, chemical composition and colloidal size, all of which can be well controlled by preparation methods, liposomes exhibit

several properties which may be useful in various applications. The most important properties are colloidal size, i.e. rather uniform particle size distributions in the range from 20 nm to 10 jum, and special membrane and surface characteristics. They include bilayer phase behav- ior, its mechanical properties and permeability, charge density, presence of surface bound or grafted polymers, or attachment of special ligands, respectively. Additionally, due to their amphiphilic character, liposomes are a powerful solubilizing system for a wide range of compounds.[28] The advances that brought about liposome-derived technologies have been recognized as some of the cornerstones of bionanotechnology. The unique advantages imparted by lipid vesicles are their diverse range of morphologies, compositions, abilities to envelope and protect many types of therapeutic biomolecules, lack of immunogenic response, low cost, and their differential release characteristics.[29]

Table 1: Advantages and disadvantages of lipososmes

S. No.	Advantage	Disadvantage
1.	Stability increased if liposomes prepared via encapsulation	Short half-life
2.	Liposomes increased the efficacy and therapeutic index of drug (actinomycin-D)	Low solubility
3.	Liposomes reduce the toxicity of the encapsulated agent (amphotericin B, Taxol)	Leakage and fusion of encapsulated drug/ molecules
4.	Liposomes help reduce the exposure of sensitive tissues to toxic drugs	Production cost is high
5.	Site avoidance effect	Fewer stables
6.	Liposomes are flexible, non-toxic, biocompatible, biodegradable, and non-immunogenic for systemic and non-completely systemic administrations	Sometimes phospholipids undergo oxidation and hydrolysis-like reaction
7.	Flexibility to couple with site-specific ligands to achieve active targeting	-

2. Therapeutic Applications of Liposomes:

When a conventional dosage form fails to provide a desired therapeutic effect, then new drug delivery systems are developed. Liposomes are among such systems which provide a superior therapeutic efficacy and safety in comparison to existing formulations. Some of the major therapeutic applications of liposomes in drug delivery include:

2.1 Site-avoidance delivery:

The cytotoxicity of anti-cancer drugs to normal tissues can be attributed to their narrow therapeutic index (TI). Under such circumstances, the TI can be improved by minimizing the delivery of drug to normal cells by encapsulating in liposomes. Free doxorubicin has a severe side effect of cardiac toxicity, but when formulated as liposomes, the toxicity was reduced without any change in the therapeutic activity.

2.2 Site-specific targeting:

Delivery of a larger fraction of the drug to the desired (diseased) site, by reducing the drug's exposure to normal tissues can be achieved by site-specific targeting. Encapsulating the drug in liposomes can be used for both active and passive targeting of drugs to achieve a safer and more efficacious therapy. On systemic administration, long-circulating immunoliposomes can recognize and bind to target cells with greater specificity. In

patients with recurrent osteosarcoma, there was an enhanced tumoricidal activity of monocytes, when muramyl peptide derivatives were formulated as liposomes and administered systemically.

2.3 Intracellular drug delivery:

Increased delivery of potent drugs to the cytosol (in which drug's receptors are present), can be accomplished using a liposomal drug delivery system. N-(phosphonacetyl)-L-aspartate (PALA) is normally poorly taken up into cells. Such drugs when encapsulated within liposomes, showed greater activity against ovarian tumor cell lines in comparison to free drugs.

2.4 Sustained release drug delivery:

Liposomes can be used to provide a sustained release of drugs, which require a prolonged plasma concentration at therapeutic levels to achieve the optimum therapeutic efficacy. Drugs like cytosine Arabinoside can be encapsulated in liposomes for sustained release and optimized drug release rate in vivo.

2.5 Intraperitoneal administration:

Tumors that develop in the intra-peritoneal (I.P.) cavity can be treated by administering the drug to the I.P. cavity. But the rapid clearance of the drugs from the I.P. cavity results in a minimized concentration of drugs at the diseased site. However, liposomal encapsulated drugs have a lower clearance rate, when compared to free drugs and can provide a maximum fraction of the drug in a prolonged manner to the target site.

2.6 Immunological adjuvants in vaccines:

Immune response can be enhanced by delivering antigens encapsulated within liposomes. Depending on the lipophilicity of antigens, the liposome can accommodate antigens in the aqueous cavity or incorporate them within the bilayers. To enhance the immune response to diphtheria toxoid, liposomes were first used as immunological adjuvants.

2.7 Liposomes in anticancer therapy:

Numerous or various types of liposome formulations of numerous anticancer agents were shown to be less toxic than the free drug. Anthracyclines are drugs that stop the growth of dividing cells by intercalating into the DNA and, thus, kill mainly rapidly dividing cells. These cells are not only in tumors but are also in hair, gastrointestinal mucosa, and blood cells; therefore, this class of drug is very toxic. The most used and studied is Adriamycin (commercial name for doxorubicin HCl; Ben Venue Laboratories, Bedford, Ohio). In addition to the above-mentioned acute toxicities, its dosage is limited by its increasing cardiotoxicity. Numerous diverse formulations were tried. In most cases, the toxicity was reduced to about 50%.

These include both acute and chronic toxicities because liposome encapsulation reduces the delivery of the drug molecules to those tissues. For the same reason, the efficiency was in many cases compromised due to the reduced bioavailability of the drug, especially if the tumor was not phagocyte or located in the organs of the mononuclear phagocyte system. In some cases, such as systemic lymphoma, the effect of liposome encapsulation showed enhanced efficacy due to the continued release effect, i.e., longer presence or long time of therapeutic concentrations in the circulation, while in several other cases, the sequestration of the drug into tissues of mononuclear phagocyte system reduced its efficacy. Applications in man showed, in general, reduced toxicity and better tolerability of administration with not too encouraging efficacy. Several different formulations are in different phases of clinical studies and show mixed results which are very useful.[17-21]

3. Stability of Liposomes:

During the development of liposomal drug products, the stability of the developed formulation is of major consideration. The therapeutic activity of the drug is governed by the stability of the liposomes right from the manufacturing steps to storage to delivery. A stable dosage form maintains the physical stability and chemical integrity of the active

molecule during its developmental procedure and storage. A well-designed stability study includes the evaluation of its physical, chemical, and microbial parameters along with the assurance of the product's integrity throughout its storage period. Hence a stability protocol is essential to study the physical and chemical integrity of the drug product in its storage.

3.1 Physical stability:

Liposomes are bilayered vesicles that are formed when phospholipids are hydrated in water. The vesicles obtained during this process are of different sizes. During its storage, the vesicles tend to aggregate and increase in size to attain a thermodynamically favorable state. During storage, drug leakage from the vesicles can occur due to fusion and breaking of vesicles, which deteriorates the physical stability of the liposomal drug product. Hence morphology, size, and size distribution of the vesicles are important parameters to assess the physical stability. To monitor this, a variety of techniques like light scattering and electron microscopy can be used to estimate the visual appearance (morphology) and size of the vesicles.

3.2 Chemical stability:

Phospholipids are chemically unsaturated fatty acids that are prone to oxidation and hydrolysis, which may alter the stability of the drug product. Along with this, pH, ionic strength, solvent system, and buffered species also play a major role in maintaining a liposomal formulation. Indeed chemical reactions can be induced even by light, oxygen, temperature, and heavy metal ions. Oxidation deterioration involves the formation of cyclic peroxides and hydroxy peroxidases due to the result of free radical generation in the oxidation process. Liposomes can be prevented from oxidative degradation by protecting them from light, by adding anti-oxidants such as alpha–tocopherol or butylated hydroxyl toluene (BHT), producing the product in an inert environment (presence of nitrogen or Argon), or by adding EDTA to remove trace heavy metals. Hydrolysis of the ester bond at the carbon position of the glycerol moiety of phospholipids leads to the formation of lyso-phosphatidylcholine (lysoPC), which enhances the permeability of the liposomal contents. Hence, it becomes necessary to control the limit of lysoPC within the liposomal drug product. This can be achieved by formulating liposomes with phosphatidylcholine free from lysoPC.

3.3 *In vivo* behavior of liposomes:

During the optimization of liposomal formulation, various physicochemical parameters are altered in order to achieve the desired bio-distribution and cellular uptake of drugs. Those parameters which affect the *in vivo* (biological) performance of liposomes are described below.

3.3.1 Liposome size:

The size of the vesicle governs the in vivo fate of liposomes because it determines the fraction cleared by RES. The rate of uptake of liposomes by RES increases with the vesicle size. Liposomes larger than 0.1μm are taken up (opsonized) more rapidly by RES when compared to liposomes smaller than 0.1μm. The size of the vesicle also determines the extravasations of liposomes. Tumor capillaries are more permeable than normal capillaries. Due to such leaky vasculature, fluids along with small-sized liposomes can pass through the gaps leading to increased accumulation of drug-loaded liposomes in the tumor tissue. The difference between intravascular hydrostatic and interstitial pressure acts as a driving force for the extravasations of small-sized liposomes.

3.3.2 Surface charge:

The lipid–cell interaction can be governed by the nature and density of charge on the liposome surface. Charging the lipid composition can alter the nature and charge of the liposome. Lack of charge in the SUV liposomes can lead to their aggregation and thereby reducing the stability of the liposome; whereas, the interaction of neutrally charged liposomes with the cell is almost negligible. High electrostatic surface charge on the liposome may provide useful results in promoting lipid–cell interaction. Negatively charged density influences the extent of lipid–cell

interactions and increases the intracellular uptake of liposomes by target cells. But positively charged liposomes are cleared more rapidly after systemic administration. Unlike negatively charged liposomes, cationic liposomes deliver the contents to cells by fusion with the cell membrane.

3.3.3 Surface hydration:

Liposomes with hydrophilic surface coatings are less prone to opsonization, hence reducing their uptake by RES cells. This can be attributed to the hydrophilic surface coating, which reduces the interaction of liposomes with cell and blood components. These sterically stabilized liposomes are more stable in the biological environment and exhibit high circulation half-lives when compared to liposomes coated with hydrophobic coatings. Monogangliosides, hydrogenated phosphatidyl inositol, and polyethylene glycol are some of the hydrophilic groups responsible for the steric stabilization of liposomes.

3.3.4 Bilayer fluidity:

Lipid exists in different physical states above and below the phase transition temperature (Tc). They are rigid and well-ordered below Tc but are in the fluid-like liquid–crystalline state above Tc indicating the phase transition temperatures of various phospholipids. Liposomes with low Tc (less than 37°C) are fluid-like and are prone to leakage of the drug content at physiological temperature. But, the liposomes with high Tc (greater than 37°C) are rigid and less leaky at physiological temperature. The phase transition temperature also governs the liposomal cell interaction. Liposomes with low Tc lipids have a high extent of uptake by RES when compared to those with high Tc lipids. The incorporation of cholesterol in the bilayer can decrease the membrane fluidity at a temperature greater than the phase transition temperature, which gives stability to liposomes.[11-15]

4. Conclusion:

Liposomes have been effectively used as an effective drug delivery mechanism for treating a variety of ailments, from the treatment of cancer to the management of chronic pain. Water-insoluble, poorly bioavailable, and extremely toxic drugs' pharmacokinetics and pharmacodynamics were improved by the biocompatible, biodegradable, and low immunogenicity liposome formulation. To get beyond their early restrictions, liposomes underwent several changes to their composition and production method. More than 500 liposomal formulations are now undergoing various stages of clinical research. A few liposome formulations are already available on the market with approval to treat a variety of disorders. So, The study of liposomes will become a more sophisticated and dependable platform for the creation of more beneficial bioproducts, particularly in the fields of public health and medical diagnostics, as other medical technologies evolve.

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