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LIPOSOMES: A NOVEL APPROACH AS A CARRIER

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ABSTRACT

Liposomes, sphere-shaped vesicles consisting of one or more phospholipid bilayers, were first described in the mid-60s. Today, they are a very important tool in various scientific disciplines. Among several talented new drug delivery systems, liposomes characterize an advanced technology to deliver active molecules to the site of action. Liposomes, which are biodegradable and essentially non-toxic vehicles, can encapsulate both hydrophilic and hydrophobic materials, and are utilized as drug carriers in drug delivery systems. As a result, numerous improvements have been made, thus making this technology potentially useful for the treatment of certain diseases in the clinics. Many liposome-based DNA delivery systems have been described, including molecular components for targeting given cell surface receptors or for escaping from the lysosomal compartment. The insight gained from clinical use of liposome drug delivery systems can now be integrated to design liposomes that can be targeted on tissues, cells or intracellular compartments with or without expression of target recognition molecules on liposome membranes. The success of liposomes as drug carriers has been reflected in a number of liposome-based formulations, which are commercially available or are currently undergoing clinical trials.

KEYWORDS: Liposomes; delivery systems; drug; target.

INTRODUCTION

New drug development is an expensive and incurs a great amount of time. The average cost for developing a new chemical entity is much more than the development of novel drug delivery system (NDDS). Such new chemical entity may take not less than ten years for their

commercial use. But developing a novel drug delivery system is rather a no time consuming and an economic process. One of the novel drug delivery system is liposome. Liposomes are the microscopic vesicles composed of one or more concentric lipid bilayers separated by water or aqueous buffer compartments with diameter ranging from 80 nm to 10 micron.

Liposomes are a form of vesicles that consist either of many, few or just one phospholipid bilayers. The polar character of the liposomal core enables polar drug molecules to be encapsulated. Amphiphilic and lipophilic molecules are solubilized within the phospholipid bilayer according to their affinity towards the phospholipids.[1] Liposomes are artificial vesicles comprised of lipid and aqueous compartments where the lipid exists in the bilayer form. Such vesicles can be composed solely of phospholipids or in combination with other amphipathic molecules such as sterols, long chain organic bases or acids. When phospholipids are suspended in an excess of aqueous solution they spontaneously form multilamellar concentric bilayers with lipid layers separated by layers of aqueous medium. Water soluble substances such as drugs, proteins, nucleic acids and dyes, present in the aqueous phase during the formation of liposomes, can be encapsulated into the aqueous compartments of the vesicles (fig. 1). This unique property of liposomes has made them a versatile tool for an increasing number of studies in biology and medicine. The earliest suggestion of a therapeutic potential for liposomes was for its possible application as carriers of enzymes and drugs *in vivo* in the therapy of various metabolic and physiological disorders.[1] The concept behind the use of liposomes as carriers of drugs and macromolecules was mainly related to an expected protection of the encapsulated molecules in the blood stream. On the contrary, it was found that liposome encapsulation of antigens resulted in elevated antibody titres in comparison to the free antigen.[2] This interesting observation opened the field for the study of the immunopotentiating properties of liposomes.

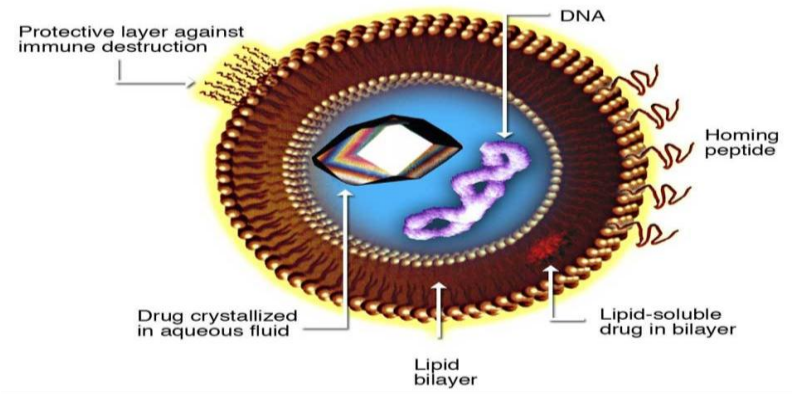

Fig. 1: Liposome for Drug delivery.

Classification of Liposomes

Liposomes can be classified either on the basis of their structural properties or on the basis of the preparation method used. These two classification system are in principle, independent of each other. The parameters for the first type of the classification are mention in the table 1. Dependent on the selection of lipids, the preparation technique, and preparation conditions, liposomes can vary widely in size, number, position of lamellae. These parameters influence the behaviour of liposomes both in vivo and in vitro. Classification based on method of liposome preparation is mentioned in the table 2.

Table 1: Liposome classification based on structural features

Liposome Abbreviation	Liposomes
MLV	Multilamellar large vesicles
OLV	Oligolamellar vesicles
UV	Unilamellar vesicles
SUV	Small unilamellar vesicles
MUV	Medium sized unilamellar vesicles
LUV	Large unilamellar vesicles
GUV	Giant unilamellar vesicles
MVV	Multivesicular vesicles

Table 2: Liposome classification based on method of liposome preparation

Liposome Abbreviation	Liposomes
REV	Single or oligolamellar vescile made by reverse phase evaporation method
MLV / REV	Multilamellar vesicles made by reverse phase evaporation method
SPLV	Stable plurilamellar vesicles
FATMLV	Frozen and thawed MLV
VET	Vesicles prepared by extrusion method
FUV	Vesicles prepared by fusion
FPV	Vesicles prepared by french press
DRV	Dehydration- rehydration vesicles
BSV	Bubblesomes

Mechanism of Liposome Formation

Liposomes are vesicular structures consisting of hydrated bilayers. Liposomes structures used for pharmaceutical purposes consist of a phospholipid backbone. But other classes of molecules can form bilayer based vesicular structures as well. On the other hand not all the hydrated phospholipids form bilayer structures. Other forms of self aggregation such as inverted hexagonal phases or micelles with completely different properties can occur. The common feature that all bilayer forming compounds share is their amphilicity. They have

defined polar and nonpolar regions. In water the hydrophobic regions tend to self aggregate and the Polar Regions tend to be in contact with the water phase. Israelachvili and coworkers defined critical packing parameter p by

$p = v/ a_0 l_c$

Where v is the molecular volume of the hydrophobic part, a_0 is the optimum surface area per molecule at the hydrocarbon water interface and l_c is the critical half thickness for the hydrocarbon region which must be less than the maximum length of the extended lipid chains. For $p < 1/3$, spherical micelles are formed. In this category fall, single chain lipids with large head group areas. For example: lysophosphatidylcholine. For ($1/3 < p < ½$) globular or cylindrical micelles are formed. Double chain "fluid state" lipids with large head area ($1/2 < p < 1$) form bilayers and vesicles. This occurs also with double chain "gel state" lipids with small head groups and for ($p \sim 1$, $p > 1$) inverted structures such as the inverted hexagonal phase can be observed. An additional condition required for bilayer formation is that the compound can be classified as a nonsoluble swelling ampiphile.[3]

Liposomes in drug delivery: evolution

One of the drawbacks of the use of liposomes is the fast elimination from the blood and capture of the liposomal preparations by the cells of the Reticulo-Endothelial System, primarily in the liver. A number of developments have aimed to reduce this problem.

Immunoliposomes

To increase liposomal drug accumulation in the desired tissues and organs, the use of targeted liposomes with surface-attached ligands capable of recognizing and binding to cells of interest has been suggested. Immunoglobulins (Ig) of the IgG class and their fragments are the most widely used targeting moieties for liposomes, which can be attached to liposomes, without affecting liposomal integrity or the antibody properties, by covalent binding to the liposome surface or by hydrophobic insertion into the liposomal membrane after modification with hydrophobic residues.[4] Still, despite improvements in targeting efficacy, the majority of immunoliposomes accumulate in the liver as a consequence of insufficient time for the interaction between the target and targeted liposome. Better target accumulation can be expected if liposomes can be made to remain in the circulation long enough.

Long-circulating liposomes

Different methods have been suggested to achieve long circulation of liposomes in vivo, including coating the liposome surface with inert, biocompatible polymers, such as PEG, which form a protective layer over the liposome surface and slow down liposome recognition by opsonins and therefore subsequent clearance of liposomes.[5,6] Long-circulating liposomes are now being investigated in detail and are widely used in biomedical in vitro and in vivo studies; they have also found their way into clinical practice.[7,8] An important feature of protective polymers is their flexibility, which allows a relatively small number of surface-grafted polymer molecules to create an impermeable layer over the liposome surface.[9,10] Long-circulating liposomes demonstrate dose-independent, non-saturable, log-linear kinetics and increased bioavailability.[11]

Long-circulating immunoliposomes

The further development of liposomal carriers involved the attempt to combine the properties of long-circulating liposomes and immunoliposomes in one preparation.[12-14] Early experiments have been performed by simple co-immobilization of an antibody and PEG on the surface of the same liposome, although the protective polymer can create steric hindrances for target recognition with the targeting moiety.[12] To achieve better selectivity of PEG-coated liposomes, it is advantageous to attach the targeting ligand via a PEG spacer arm, so that the ligand is extended outside of the dense PEG brush, which reduces steric hindrance of binding to the target. Currently, various advanced technologies are used, and the targeting moiety is usually attached above the protecting polymer layer, by coupling it with the distal water-exposed terminus of activated liposome-grafted polymer molecule.[13,15]

Advantages

Some of the advantages of liposome are as follows:

- Provides selective passive targeting to tumor tissues (Liposomal doxorubicin).
- Increased efficacy and therapeutic index.
- Increased stability via encapsulation.
- Reduction in toxicity of the encapsulated agents.
- Site avoidance effect.
- Improved pharmacokinetic effects (reduced elimination, increased circulation life times).
- Flexibility to couple with site specific ligands to achieve active targeting.[16]

Choice of method

The correct choice of liposome preparation method depends on the following parameters:

- the physicochemical characteristics of the material to be entrapped and those of the liposomal ingredients;
- the nature of the medium in which the lipid vesicles are dispersed;
- the effective concentration of the entrapped substance and its potential toxicity;
- additional processes involved during application/delivery of the vesicles;
- optimum size, polydispersity and shelf-life of the vesicles for the intended application and
- batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.[17,18]

All the methods of preparing the liposomes involve four basic stages:

- Drying down lipids from organic solvent
- Dispersing the lipid in aqueous media
- Purifying the resultant liposome
- Analyzing the final product

Methods of liposomes preparations

The following methods are used for the preparation of liposome:

1. Passive loading techniques
2. Active loading technique

Passive loading techniques include three different methods

1. Mechanical dispersion method
2. Solvent dispersion method
3. Detergent removal method (removal of non encapsulated material) [19,20]

Mechanical dispersion method

The following are types of mechanical dispersion methods

- Sonication
- French pressure cell: extrusion
- Freeze-thawed liposomes
- Lipid film hydration by hand shaking, non-hand. shaking or freeze drying
- Micro-emulsification
- Membrane extrusion

- Dried reconstituted vesicles[19,20]

Sonication

Sonication is perhaps the most extensively used method for the preparation of SUV. Here, MLVs are sonicated either with a bath type sonicator or a probe sonicator under a passive atmosphere. The main disadvantages of this method are very low internal volume/encapsulation efficacy, possible degradation of phospholipids and compounds to be encapsulated, elimination of large molecules, metal pollution from probe tip, and presence of MLV along with SUV.[19]

There are two sonication techniques

a) Probe sonication: The tip of a sonicator is directly engrossed into the liposome dispersion. The energy input into lipid dispersion is very high in this method. The coupling of energy at the tip results in local hotness; therefore, the vessel must be engrossed into a water/ice bath. Throughout the sonication up to 1 h, more than 5% of the lipids can be deesterified. Also, with the probe sonicator, titanium will slough off and pollute the solution.

b) Bath sonication: The liposome dispersion in a cylinder is placed into a bath sonicator. Controlling the temperature of the lipid dispersion is usually easier in this method, in contrast to sonication by dispersal directly using the tip. The material being sonicated can be protected in a sterile vessel, dissimilar the probe units, or under an inert atmosphere.[21]

French pressure cell: extrusion

French pressure cell involves the extrusion of MLV through a small orifice.[19] An important feature of the French press vesicle method is that the proteins do not seem to be significantly pretentious during the procedure as they are in sonication.[22] An interesting comment is that French press vesicle appears to recall entrapped solutes significantly longer than SUVs do, produced by sonication or detergent removal.[23-25] The method involves gentle handling of unstable materials. The method has several advantages over sonication method.[26] The resulting liposomes are rather larger than sonicated SUVs. The drawbacks of the method are that the high temperature is difficult to attain, and the working volumes are comparatively small (about 50 mL as the maximum).[19,20]

Freeze-thawed liposomes

SUVs are rapidly frozen and thawed slowly. The short-lived sonication disperses aggregated materials to LUV. The creation of unilamellar vesicles is as a result of the fusion of SUV throughout the processes of freezing and thawing.[27-29] This type of synthesis is strongly inhibited by increasing the phospholipid concentration and by increasing the ionic strength of the medium. The encapsulation efficacies from 20% to 30% were obtained.[27]

Solvent dispersion method

Ether injection (solvent vaporization)

A solution of lipids dissolved in diethyl ether or ether-methanol mixture is gradually injected to an aqueous solution of the material to be encapsulated at 55°C to 65°C or under reduced pressure. The consequent removal of ether under vacuum leads to the creation of liposomes. The main disadvantages of the technique are that the population is heterogeneous (70 to 200 nm) and the exposure of compounds to be encapsulated to organic solvents at high temperature.[30,31]

Ethanol injection

A lipid solution of ethanol is rapidly injected to a huge excess of buffer. The MLVs are at once formed. The disadvantages of the method are that the population is heterogeneous (30 to 110 nm), liposomes are very dilute, the removal all ethanol is difficult because it forms into azeotrope with water, and the probability of the various biologically active macromolecules to inactivate in the presence of even low amounts of ethanol is high.[32]

Reverse phase evaporation method

This method provided a progress in liposome technology, since it allowed for the first time the preparation of liposomes with a high aqueous space-to-lipid ratio and a capability to entrap a large percentage of the aqueous material presented. Reverse-phase evaporation is based on the creation of inverted micelles. These inverted micelles are shaped 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 elimination of the organic solvent leads to the conversion of these inverted micelles into viscous state and gel form. At a critical point in this process, the gel state collapses, and some of the inverted micelles were disturbed. The excess of phospholipids in the environment donates to the formation of a complete bilayer around the residual micelles, which results in the creation of liposomes. Liposomes made by

reverse phase evaporation method can be made from numerous lipid formulations and have aqueous volume-to-lipid ratios that are four times higher than hand-shaken liposomes or multilamellar liposomes.[20,21]

Briefly, first, the water-in-oil emulsion is shaped by brief sonication of a two-phase system, containing phospholipids in organic solvent such as isopropyl ether or diethyl ether or a mixture of isopropyl ether and chloroform with aqueous buffer. The organic solvents are detached under reduced pressure, resulting in the creation of a viscous gel. The liposomes are shaped when residual solvent is detached during 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 Na Cl. The method has been used to encapsulate small, large, and macromolecules. The main drawback of the technique is the contact of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the breakage of DNA strands or the denaturation of some proteins.[33] Modified reverse phase evaporation method was presented by Handa et al., and the main benefit of the method is that the liposomes had high encapsulation efficiency (about 80%).[34]

Detergent removal method (removal of non-encapsulated material)

Dialysis

The detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids. As the detergent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. The detergents were removed by dialysis.[35,37] A commercial device called Lipo Prep (Diachema AG, Switzerland), which is a version of dialysis system, is obtainable for the elimination of detergents. The dialysis can be performed in dialysis bags engrossed in large detergent free buffers (equilibrium dialysis).[38]

Detergent (cholate, alkyl glycoside, Triton X-100) removal of mixed micelles (absorption) Detergent absorption is attained by shaking mixed micelle solution with beaded organic polystyrene adsorbers such as XAD-2 beads (SERVA Electrophoresis GmbH, Heidelberg, Germany) and Bio-beads SM2 (Bio-Rad Laboratories, Inc., Hercules, USA). The great benefit of using detergent adsorbers is that they can eliminate detergents with a very low CMC, which are not entirely depleted.

Gel-permeation chromatography

In this method, the detergent is depleted by size special chromatography. Sephadex G-50, Sephadex G-l 00 (Sigma-Aldrich, MO, USA), Sepharose 2B-6B, and Sephacryl S200-S1000 (General Electric Company, Tehran, Iran) can be used for gel filtration. The liposomes do not penetrate into the pores of the beads packed in a column. They percolate through the inter-bead spaces. At slow flow rates, the separation of liposomes from detergent monomers is very good. The swollen polysaccharide beads adsorb substantial amounts of amphiphilic lipids; therefore, pretreatment is necessary. The pre-treatment is done by pre-saturation of the gel filtration column by lipids using empty liposome suspensions.

New Large-Scale Liposome Technique

Since industrial scale production of liposomes has become reality, the range of liposome preparation methods has been extended by a number of techniques such as Heating Method, Spray drying, Freeze Drying, Super Critical Reverse Phase Evaporation (SCRPE), and several modified ethanol injection techniques which are increasingly attractive.

Heating Method

A new method for fast production of liposomes without the use of any hazardous chemical or process has been described.[39] This method involves the hydration of liposome components in an aqueous medium followed by the heating of these components, in the presence of glycerol (3% v/v), up to 120^0C. 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. Temperature and mechanical stirring provide adequate energy for the formation of stable liposomes. Reza Mozafari et al. confirmed by TLC that no degradation of the used lipids occurred at the above mentioned temperatures.[40] The particle size can be controlled by the phospholipid nature and charge, the speed of the stirring and the shape of the reaction vessel. Otherwise, employment of heat abolishes the need to carry out any further sterilisation procedure reducing the time and cost of liposome production.

Spray-Drying

Since spray-drying is a very simple and industrially applicable method, the direct spray-drying of a mixture of lipid and drug was applied in the preparation of liposomes. The spray-drying process is considered to be a fast single-step procedure applied in the nanoparticles formulation. Hence, liposomes were prepared by suspending lecithin and mannitol in

chloroform. The mixture was sonicated for 8 min (bath sonicator) and subjected to spray-drying on a Buchi 190 M Mini Spray Dryer. The spray-drying conditions were as follows: inlet and outlet temperatures were 120^0C and 80^0C, respectively; airflow rate was 700 NI/hr; and the flow rate was 1000 ml/hr. The dried product was hydrated with different volumes of phosphate buffered saline (PBS; pH 7.4) by stirring for 45 min.[41] The main factor influencing the liposomal size was the volume of aqueous medium used for hydration of the spray-dried product. However, mannitol plays an important role in increasing the surface area of the lipid mixture, enabling successful hydration of the spray-dried product.

Freeze Drying

This new method was described for the preparation of sterile and pyrogen-free submicron narrow sized liposomes.[42,43] It is based on the formation of a homogenous dispersion of lipids in water-soluble carrier materials. Liposome-forming lipids and water-soluble carrier materials such as sucrose were dissolved in tert-butyl alcohol/water cosolvent systems in appropriate ratios to form a clear isotropic monophase solution. Then the monophase solution was sterilized by filtration and filled into freeze-drying vials. In recent study, a laboratory freeze drier was used and freeze-drying process was as follows: freezing at -40^0C for 8 h; primary drying at -40^0C for 48 h and secondary drying at 25^0C for 10 h,[42] The chamber pressure was maintained at 20 Pascal during the drying process. On addition of water, the lyophilized product spontaneously forms homogenous liposome preparation. After investigation of the various parameters associated with this method it is found that the lipid/carrier ratio is the key factor affecting the size and the polydispersity of the liposome preparation.[42] Therefore, TBA/water cosolvent system was used for economy concerns.

Super Critical Reverse Phase Evaporation (SCRPE)

The SCRPE is a one-step new method that has been developed for liposomes preparation using supercritical carbon dioxide.[44] This method allowed aqueous dispersions of liposomes to be obtained through emulsion formation by introducing a given amount of water into a homogeneous mixture of supercritical carbon dioxide/LR-dipalmitoylphosphatidylcholine/ethanol under sufficient stirring and subsequent pressure reduction. Transmission electron microscopy observations revealed that vesicles are large unilamellar with diameters of 0.1– 1.2 µm.[44] The trapping efficiency of these liposomes indicated more than 5 times higher values for the water-soluble solute compared to multilamellar vesicles prepared by the Bangham method. The trapping efficiency for an

oilsoluble substance, the cholesterol, was about 63%. Results showed that the SCRPE is an excellent technique that permits one-step preparation of large unilamellar liposomes exhibiting a high trapping efficiency for both water-soluble and oil-soluble compounds.[45, 46]

Modified Ethanol Injection Method

Novel approaches based on the principle of the ethanol injection technique such as the microfluidic channel method,[47-49] the crossflow injection technique,[50-53] and the membrane contactor method[54] were recently reported for liposome production.

The Crossflow Injection Technique

The concept of continuous crossflow injection is a promising approach as a novel scalable liposome preparation technique for pharmaceutical application. Wagner et al. used a cross flow injection module made of two tubes welded together forming a cross technique.[50-53] At the connecting point, the modules were adapted with an injection hole. The influencing parameters such as the lipid concentration, the injection hole diameter, the injection pressure, the buffer flow rate, and system performance were investigated.[50] A minimum of buffer flow rate is required to affect batch homogeneity and strongly influencing parameters are lipid concentration in combination with increasing injection pressures. After exceeding the upper pressure limit of the linear range, where injection velocities remain constant, the vesicle batches are narrowly distributed, also when injecting higher lipid concentrations. Reproducibility and scalability data show similar results with respect to vesicle size and size distribution and demonstrate the stability and robustness of the novel continuous liposome preparation technique.[52]

Microfluidization

By using a microfluidic hydrodynamic focusing (MHF) platform, Jahn et al. generated liposomes by injecting the lipid phase and the water phase into a microchannel.[48] Microfluidic flow is generally laminar due to the small channel dimensions and relatively low flow rates. Well-defined mixing is then obtained by interfacial diffusion when multiple flow streams are injected in a microchannel. The size of the liposomes was mainly controlled by changing the flow rate.[47]

Membrane Contactor

Recently, Jaafar-Maalej et al. applied the ethanol injection technique while using a membrane contactor for large scale liposomes production. In this method, a lipid phase (ethanol,

phospholipid and cholesterol) was pressed through the membrane with a specified pore size. Nitrogen gas at pressure below 5 bar was sufficient for passing the organic phase through the membrane. At the same time, the aqueous phase flew tangentially to the membrane surface and swept away the formed liposomes within the membrane device. The new process advantages are the design simplicity, the control of the liposome size by tuning the process parameters and the scaling-up abilities.[54] As a result, these techniques lead from the conventional batch process to potential large scale continuous procedures.

Handling of Liposomes

- The lipids used in the preparation of liposomes are unsaturated and hence susceptible to oxidation.
- Also volatile solvents such as chloroform which are used will tend to evaporate from the container.
- Thus liposomes must be stored in an inert atmosphere of nitrogen, and in the dark, in glass vessels with a securely fastened cap.

DISCUSSION AND APPLICATIONS

Liposomes are used for drug delivery due to their unique properties. A liposome encapsulates a region on aqueous solution inside a hydrophobic membrane; dissolved hydrophilic solutes cannot readily pass through the lipids. Hydrophobic chemicals can be dissolved into the membrane and in this way liposome can carry both hydrophobic molecules and hydrophilic molecules. To deliver the molecules to sites of action, the lipid bilayer can fuse with other bilayers such as the cell membrane, 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 (indiscriminately) delivered past the lipid bilayer.

Liposomes as carriers of protein and peptides

During the past two decades native, biologically active compounds of protein/peptide origin, such as enzymes, peptide hormones, cytokines and so on, have become drugs of choice for the treatment of various diseases.

Liposomal proteins

From a clinical point of view, the potential ability of liposome-encapsulated enzymes to enter the cytoplasm or lysosomes of live cells is of crucial importance for the treatment of inherited

diseases caused by the abnormal functioning of some intracellular enzymes (that is, the lysosomal storage diseases) and cancer.[55]

A very interesting approach to the use of liposomal enzymes is their application to Antibody-Directed Enzyme Prodrug Therapy (ADEPT) based on the on-site activation of chemically modified inactive phospholipid derivatives of various anticancer and antiviral agents. The application of phospholipid prodrugs incorporated into liposome membranes brings several benefits.[56] The efficiency of prodrug incorporation is high; prodrugs do not leak from the liposome into the aqueous phase; drugs are protected against metabolic degradation; and long-lasting therapeutic drug levels can be achieved. The achieve the specific generation of active cytotoxic molecules from inactive prodrugs in the vicinity of tumour cells, a conjugate of a tumour-specific antibody with an enzyme responsible for the conversion of a prodrug into the active drug is targeted towards tumour. To increase the efficiency of the required enzyme in the tumour, rather than just 'straight' antibody-enzyme conjugates, immunoliposomes have been loaded with the required enzyme (immuno-enzymosomes).[57]

Liposomes in gene delivery

The use of liposomes for gene delivery applications is a huge area that will be only briefly addressed here. Although viral systems are currently the most common means for DNA delivery, non-viral systems have also been developed. Cationic lipid-based liposomes[58] are easy to prepare, reasonably cheap and nonimmunogenic. Many of the finer features of these delivery systems and mechanisms remain insufficiently understood, and so recent studies in this popular area have tended to concentrate on structure, function, structure–activity relationships, detailed mechanisms of liposome-mediated gene delivery, and improved efficiency of transfection.[59] To combine the longevity of liposomal preparations with efficient DNA delivery, pre-condensed DNA has been encapsulated into PEGylated cationic liposomes.[60] Recently, the use of polycationic liposomes for gene delivery has been proposed — that is, liposomes modified by cetylated polyethylene imine, which anchors in the membrane via cetyl residues and binds DNA via positive charges. Such liposomes demonstrate good loading with DNA and high transfection efficacy.[61]

Drug targeting

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. By this approach the otherwise preferential distribution of liposomes into the Reticulo-Endeothelial system (RES; liver, spleen and bone marrow) is averted or minimized.

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.

Treatment of human immunodeficiency virus (HIV) infections

Several antiretroviral nucleotide analogues have been developed for the treatment of patients suffering from the Acquired Immunodeficiency Syndrome (AIDS). These include antisense oligonucleotide, which is a new antiviral agent that has shown potential therapeutic application against HIV-1.

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 cephalosporin are sensitive to the degradative action of J-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.

Agro-food industry

liposomes encapsulated biocides have shown superior action due to prolonged presence of the fungicides, herbicides or pesticides at reduced damage to other life forms.[62] Liposome surface can be made sticky so that they remain on the leafs for longer times and they do not wash into the ground. In these applications inexpensive liposomes produced from synthetic lipids are used.

The sustained release system concept can be used in various fermentation processes in which the encapsulated enzymes can greatly shorten fermentation times and improve the quality of the product. This is due to improved spatial and temporal release of the ingredient (s) as well as to their protection in particular phases of the process against chemical degradation. A classical example is cheese making. The first serious attempts to decrease the fermentation

time using cell-wall-free bacterial extracts were encouraging enough to stimulate efforts to improve enzyme presentation. After preliminary studies in which liposome systems were optimized the cheese ripening times can be shortened by 30–50% [63-65]. This means a substantial economic profit knowing that ripening times of some cheeses, such as Cheddar, say, are about one year during which they require well controlled conditions. In addition, due to the better dispersal of the enzymes the texture of cheeses was even and bitterness and inconsistent flavor due to the proteolysis of enzymes in the early phase of fermentation was much improved.[63,64]

CONCLUSION

Liposomes are one of the unique drug delivery system, which can be of potential use in controlling and targeting drug delivery. Liposomes are administrated orally, parenterally and topically as well as used in cosmetic and hair technologies, sustained release formulations, diagnostic purpose and as good carriers in gene delivery. Nowadays, liposomes are used as versatile carriers for targeted delivery of drug. Further advances in liposome research have been able to allow liposomes to avoid detection by the body's immune system, specifically, the cells of Reticulo-Endothelial System (RES). These liposomes are known as "stealth liposomes", and are constructed with PEG (Polyethylene Glycol) studding the outside of the membrane. The PEG coating, which is inert in the body, allows for longer circulatory life for the drug delivery mechanism.

Finally, the major challenge for liposome is the large scale production method. Pharmaceutically acceptable procedures are those that can be easily scaled to larger batch sizes and economically feasible. However, unlike the classical pharmaceutical dosage forms (tablets, capsules, suppository etc.) which are produced in large batch sizes, liposome based drugs even those already in the market are produced in small size batches and thus are costly for the manufacturers. Scale-up process to larger size batches is often a monumental task for the process development scientists.

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Conflict of Interest statement

There is no conflict of interest.

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