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Nanoliposome: An alternative approach for drug delivery system

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REVIEW ARTICLE

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ABSTRACT

Nanoliposome, or submicron bilayer lipid vesicle, is a new technology for the encapsulation and delivery of bioactive agents. The list of bioactive material that can be incorporated to nanoliposomes is huge, ranging from pharmaceuticals to cosmetics and nutraceuticals. Nanoliposomes have been used to improve the therapeutic index of new or established drugs by modifying drug absorption, reducing metabolism, prolonging biological half-life and reducing toxicity. The sole characteristic of nanoliposomes is their ability to compartmentalize and solubilize both hydrophilic and hydrophobic materials. This sole characteristic, coupled with biocompatibility and biodegradability make nanoliposomes very attractive as drug delivery vehicles. This review article intends to provide an overview of liposomes and nanoliposomes their properties, preparation methods and evaluation parameters. Also it explains various applications of nanoliposomes in nanotherapy including diagnostics, targeted cancer, gene therapy, cosmetics and nutraceuticals.

Keywords: Liposome, Nanoliposome, Lipidic carrier, Nanotherapy.

INTRODUCTION

In recent decades, significant efforts has been devoted to develop nanotechnology and nanomedicine for drug delivery and controlled release for small molecular drugs as well as macromolecules such as proteins, peptides or genes have received extensive attention. The nanoparticles were developed as carriers for vaccines and anticancer drugs. Simultaneously, the nanoparticles for oral, ophthalmic and parenteral delivery were investigated (Tiyaboonchai, 2003).

NANOPARTICLES

Nanoparticles are defined as particulate dispersions or solid particles composed of synthetic or semi synthetic polymer with a size in the range of 10-1000nm. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticles matrix. Nanoparticles are of different types depending upon the method of preparation i.e. Nanoparticles, nanospheres or nanocapsules can be obtained. Nanocapsules are the systems in which drug is restricted to a hollow space surrounded by a unique polymer membrane. Nanospheres are submicron size colloidal particles with a therapeutic agent either entrapped in the polymer matrix or bound on to the surface. The nanospheres were classically 100 to 150 nanometers in diameter with the drug entrapped into the polymer matrix (Mohanraj et al., 2006).

NANOLIPOSOME

The term nanoliposome has recently been introduced to exclusively refer to nanoscale lipid vesicles. Nanoliposomes have the same physical, structural, thermodynamic properties manufacturing and mechanism of formation as the liposomes. The underlying mechanism for the formation of liposomes and nanoliposomes is basically the hydrophilic-hydrophobic interaction between phospholipids and water molecules (Khosravi-Darani, Mozafari., 2010). Nanoliposome, or submicron bilayer lipid vesicle, is a new technology for the encapsulation and delivery of bioactive agents. The lists of bioactive material that can be integrated to nanoliposomes are vast, ranging from pharmaceuticals to cosmetics and nutraceuticals. Due to the nature of biocompatibility and biodegradability, along with their nanosize,

nanoliposomes have potential applications in an enormous range of fields, including nanotherapy, cosmetics, food technology and agriculture. Nanoliposomes are able to enhance the performance of bioactive agents by improving their solubility and bioavailability, in vitro and in vivo stability, as well as preventing their unwanted interactions with other molecules (Mozafari., 2010). Nanoliposomes can also provide slow release of an encapsulated drug, resulting in sustained exposure to the site of action and enhanced efficacy. Usually hydrophilic drugs can be incorporated in aqueous compartment and lipophilic drugs are incorporated in the phospholipid layer (Patel et al., 2009). On the other hand unlike liposome nanoliposome does not undergo rapid degradation and clearance by liver macrophages. For the targeted drug delivery, nanoliposome plays a vital role. It can be used for passive targeting or active targeting (Kumar et al., 2010).

By active targeting liposomes directly go to the targeted organs or tissues, and release drug for a prolonged period of time, so that the normal cells are not affected and only the diseased cells are affected (Arab et al., 2012). Among all the nanomedicine platforms, liposomes have demonstrated one of the most established nanoplatforms, with several FDA-approved formulations because of their size, biodegradability, hydrophobic and hydrophilic character, low toxicity and immunogenicity (Jie et al., 2011). The use of nanoliposomes could allow high loading efficiency and monodisperse size distribution (Park., 2007).

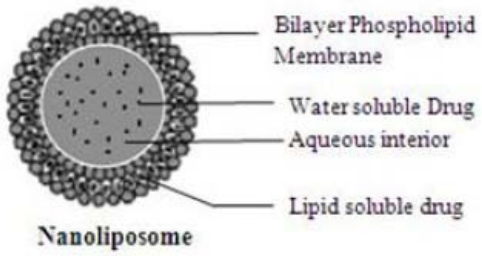

Figure 1. Basic structure of nanoliposomes.

CHEMICAL CONSTITUENT OF NANOLIPOSOME

The main chemical ingredients of nanoliposomes are lipid and/or phospholipid molecules. Phospholipids are amphiphilic, possessing both hydrophilic (water soluble) and hydrophobic (lipid soluble) properties. The head group of a phospholipid is hydrophilic and its fatty acid tail (acyl chain) is hydrophobic. Along with lipid and/or phospholipid molecules, nanoliposomes may contain other molecules such as sterols in their structure. Incorporation of sterols into nanoliposome bilayers can bring about major changes in the properties of these vesicles. The most widely used sterol in the manufacture of the lipid vesicles is cholesterol (Chol). Cholesterol does not by itself form bilayer structures, but it can be included into phospholipid membranes in very high concentrations, for example up to 1:1 or even 2:1 molar ratios of cholesterol to a phospholipid (Yeagle et al., 1990). Cholesterol is used in nanoliposome structures in order to enhance the stability of the vesicles by modulating the fluidity of the lipid bilayer. In general, cholesterol modulates fluidity of phospholipid membranes by preventing crystallization of the phospholipids. This contributes to the stability of nanoliposomes and reduces the permeability of the lipid membrane to solutes. The amount of cholesterol to be used in the nanoliposomal formulations mostly depends on the proposed application. The major structural components of liposomes/ nanoliposomes are similar.

Nanoliposomes as carriers have certain advantages:

- Nanoliposome formulations enhance the solubility and/or stability of drugs in vivo for improving physicochemical characterization and subsequent increasing in-vivo kinetics and biodistribution.
- This type of drug delivery system is meant for reduced toxic effects by decreasing toxicity of drugs.
- Liposomes can be used as targeted drug delivery system at any site in the body with different formulation.
- Liposomes can show improved activity against extra cellular pathogens to conquer bacterial drug resistance.

CLASSIFICATION OF LIPOSOMES

Classification of liposomes on the basis of structure

The liposome size can vary from very small (0.025 µm) to large (2.5 µm) vesicles. (Samad., 2007). The vesicle size is a keen limitation in determining the circulation half-life of liposomes, and both size and number of bilayers affect the amount of drug encapsulation in the liposomes. On the basis of their size and number of bilayers, liposomes can also be classified into one of two categories that is a multilamellar vesicles (MLV) and an unilamellar vesicles (Mozafari., 2005). In unilamellar liposomes, the vesicle has a single phospholipid bilayer sphere enclosing the aqueous solution. In multilamellar liposomes, vesicles have an onion structure. Typically, several unilamellar vesicles will form on the inside of the other with smaller size, making a multilamellar structure of concentric phospholipid spheres separated by layers of water (Lasic, 2001).

Table 1. Vesicle types with their size and number of lipid layers.

Vesicle Types	Abbrev	Diameter Size	Number of lipid bilayers
Unilamellar vesicles	UV	All size range	One
Small unilamellar vesicles	SUV	20-100 nm.	One
Large unilamellar vesicles	LUV	>100nm.	One
Giant unilamellar vesicles	GUV	> 1μm.	One
Multilamellar vesicles	MLV	>0.5μm.	Five to twenty
Oligolamellar vesicles	OLV	0.1-1μm.	Approximately five
Multivesicular vesicles	MMV	>1μm.	Multicompartmental structure

CLASSIFICATION OF LIPOSOMES ON THE BASIS OF METHOD OF PREPARATION

Table 2. Different preparation methods and the vesicles formed by these methods.

Preparation method	Vesicle types
Single or oligo lamellar vesicle made by reverse phase evaporation method	REV
Multi lamellar vesicle made by reverse phase evaporation method	MLV-REV
Stable pluri lamellar vesicle	SPLV
Frozen and thawed multi lamellar vesicle	FATMLV
Vesicle prepared by extrusion technique	VET
Dehydration – Rehydration method	DRV

METHODS OF PREPARATION OF NANOLIPOSOME

The manufactured liposome features are directly related to the preparation method. Although liposome formation may be spontaneous, often some mechanical agitation is required.

There are a few parameters that should be considered during the method selection:

1) The physicochemical characteristics of the material to be entrapped and those of the liposomal ingredients, 2) the nature of the medium in which the liposomes are dispersed, 3) the effective concentration of the encapsulated material and its potential toxicity, 4) optimum size, polydispersity and shelf-life of the liposome and 6) batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products (Anwekar et al., 2011).

Liposome size is a critical parameter in determining the circulation half-life of liposomes in drug delivery. The amount of encapsulated drug is also related with the size and the number of bilayers of the prepared liposome.

General methods of preparation

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.

Different methods of nanoliposomes preparation:

The conventional methods for preparing nanoliposomes include solubilizing the lipids in organic solvent, drying down the lipids from organic solution, dispersion of lipids in aqueous media, purification of resultant liposomes and analysis of the final product (Kalepu Sandeep et al., 2013). Some of the commonly used methods for the preparation of nanoliposomes are described below.

Mechanical dispersion method

The following are types of mechanical dispersion methods:

a) Sonication.
b) French pressure cell: extrusion.
c) Micro- fluidizer

Solvent dispersion methods

a) Ether Injection Method
b) Ethanol Injection Method

Detergent removal method (removal of non-encapsulated material)

a) Dialysis
b) Detergent removal of mixed micelles

Sonication method

Sonication is a simple method for reducing the size of liposomes and manufacture of nanoliposomes (Jesorka, Orwar, 2008). The sonication method is based on size transformation and involves the subsequent sonication of MLVs. Transfer the MLVs either to a bath-type sonicator or a probe sonicator (Fig. 2). For probe sonication, place the tip of the sonicator in the MLV flask and sonicate the sample with 20 s ON, 20 s OFF intervals, for a total period of 10–15 min (Prabhu et al., 2010). At this stage, nanoliposomes are formed, which are mostly in the form of small unilamellar vesicles (SUV). Otherwise, nanoliposomes can be produced using a bath sonicator.

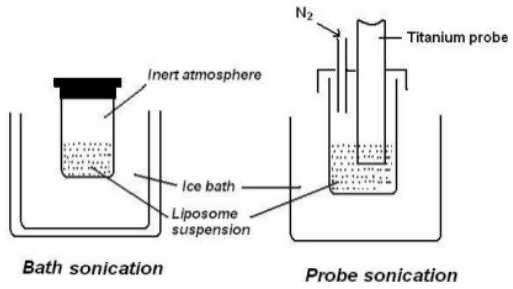

Figure 2. Different types of sonicators. A) Bath sonicator B) Probe sonicator.

Probe sonication: The tip of a sonicator is directly immersed 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; as a result, the vessel must be deep into a water/ice bath. All through the sonication up to 1 h, more than 5% of the lipids can be de-esterified. Also, with the probe sonicator, titanium will pollute the solution.

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 compare to sonication by dispersal directly using the tip. The sonicated material can be protected in a sterile vessel under an inert atmosphere.

French pressure cell (extrusion)

Extrusion is a process in which micrometric liposomes (e.g. MLV) are structurally modified to large unilamellar vesicles (LUV) or nanoliposomes depending on the pore-size of the filters used (Berger et al., 2001). Vesicles are physically extruded under pressure through polycarbonate filters of defined pore sizes. This procedure has advantages over the sonication method. In this method a variety of membrane pore sizes are available for producing liposomes in different selected size ranges, and in addition, the size distribution of the liposomes can be made fairly narrow, mainly by cycling the material through the selected-size filter several times. The membrane extrusion method also has several drawbacks in large-scale processing. For one, the pores in the membrane tend to clog up, predominantly when processing concentrated suspensions and/or when the liposome sizes are substantially greater than the membrane pore sizes. The clogged membranes cannot be cleared and replacing the filter is likely to compromise the sterility of the extrusion operation. Secondly, the membranes themselves are planar disks which must be mounted against a flat mechanical support. This harshly restricts the surface area available for extrusion, and leads to deliberate throughput. Although the problems of clogging and deliberate throughput can be overcome partially at high extrusion pressures (Himanshi et al., 2015).

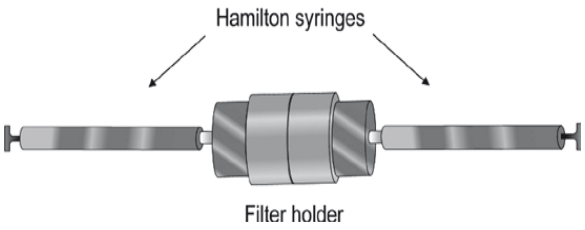

Figure 3. Extruder.

Micro-fluidization/ Micro-emulsion

This is a method of nanoliposome production without using potentially toxic solvents. The microfluidization technique using equipment called microfluidizer (Jafari et al., 2006). Microfluidization is based on the principle of dividing a pressure stream into two parts, passing each part through a fine orifice, and directing the flows at each other inside the chamber of microfluidizer (Jafari et al., 2006). Within the interaction chamber, cavitation, along with shear and impact, reduces particle sizes of the liposomes. Microfluidizer uses high pressures (up to l0, 000 psi) to direct the flow stream through microchannels toward the impingement area (Sorgi et al., 1994). The reduction in the size can be achieved by recycling of the sample. The process is reproducible and yield liposomes with high-quality aqueous phase encapsulation. Micro-fluidizer is used to formulate small vesicles from concentrated lipid concentration. The lipid can be introduced into the fluidizer as a suspension of large MLVs. The first MLVs were prepared by these were passed through a Microfluidizer at 40 psi inlet air pressure. The size varied from 150-160 nm after 25 recycles (Gaurav, Sharma., 2011).

Ethanol Injection Method: The lipid solution of ethanol is quickly injected to a excess amount of buffer and the MLVs are immediately formed. The major drawbacks of the method are the production of heterogeneous products (30-110 nm) and liposomes are very dilute. It is difficult to remove the entire 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 (Batzri and Korn, 1973).

Ether Infusion Method: The 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 major drawbacks of the method are the production of heterogeneous (70-190 nm) products and the exposure of compounds to be encapsulated to organic solvents or high temperature (Loxley., 2009).

Dialysis Method

The detergents at their critical micelle concentrations (CMC) have been used to solubilize lipids. As the deter-gent is detached, the micelles become increasingly better-off in phospholipid and lastly combine to form LUVs. The detergents were removed by dialysis. The dialysis can be per-formed in dialysis bags deep in large detergent free buffers.

Detergent removal of mixed micelles.

The absorption of detergent is attained by shaking mixed micelle solution with beaded organic polystyrene absorbers such as XAD-2 beads, and Bio-beads SM2. The main benefit of using detergent

absorbers is that they can eliminate detergents with a very low CMC, which are not entirely exhausted.

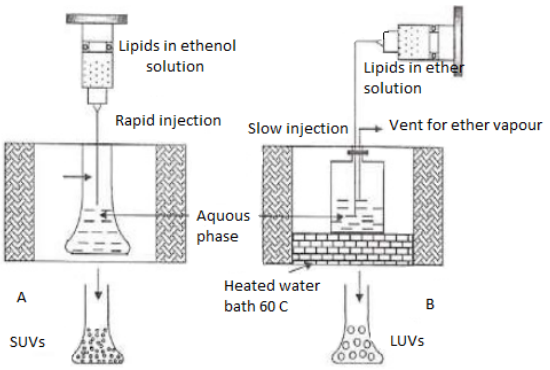

Figure 4. Sonication apparatus for Ethenol injection (A) & Ether injection (B).

CHARACTERIZATION AND EVALUATION OF NANOLIPOSOMES

The different preparation of nanoliposomes, particularly when using a new technique, characterization is required to make sure adequate quality of the product. Each and every technique has characteristic advantages and probable disadvantages. The most important parameters of nanoliposome characterization include visual appearance, size distribution, stability, Zeta potential, lamellarity and entrapment efficiency.

Visualization

Visualization can be observed by an optical microscope (phase contrast) that can detect particles size greater than 300 nm and contamination with larger particles. The size distribution of nanoliposomes is mainly determined using electron microscopy. The more newly developed microscopic technique with very high resolutions is the Scanning Probe Microscopy (SPM). One of the most applied SPM techniques are Scanning Tunnelling Microscopy (STM) and Atomic Force Microscopy (AFM). This new technology gives the possibility to view various biological and non-biological samples under air or water with a resolution up to 3A (Ozer., 2007).

Size Determination

There are different techniques are described in literature for determination of particle size and size distribution. Electron microscopic methods are generally used for establishing the morphology, size and stability of liposomes. With respect to a statistically significant analysis of size distribution of the lipid vesicles, methods such as light scattering, which measure the size of large number of vesicles in an aqueous medium that are more suitable than microscopic techniques. Either of these applied with other routine laboratory techniques, such as gel permeation chromatography, to provide a comprehensive and reliable characterization of the nanoliposomal formulations (Takeuchi et al, 1998). The most accurate method of determine size of liposome is electron microscopy because it permit to view each individual liposome and obtain exact information about profile of liposome population over the entire range of sizes. In addition more recently developed microscopic technique known as atomic force microscopy has been utilized to study liposome morphology, size, and stability.

Zeta Potential

Zeta potential is the overall charge in a lipid vesicle acquires in a particular medium. It is a measure of the magnitude of repulsion or attraction between particles in general and lipid vesicles in particular. Evaluation of the zeta potential of a nanoliposome preparation can help to predict the stability and in vivo outcome of liposomes. Understanding of the zeta potential is also useful in controlling the aggregation, fusion and precipitation of nanoliposomes, which are important factors affecting the stability of nanoliposomal formulations.

Lamellarity Determination

Lamellarity of vesicles is the number of bilayer present in liposomes which are determined by using Freeze- Fracture Electron Microscopy and P-31 Nuclear Magnetic Resosance (^{31}P NMR) analysis (Jesorka, Orwar., 2008). Additional techniques for lamellarity determination comprise electron microscopy, small angle X-ray scattering (SAXS), and also the methods that are based on the change in the visible or fluorescence upon the addition of reagents.

Entrapment Efficiency

The entrapment efficiencies of prepared nanoliposomes were determined by finding the concentration of free drug in the dispersion medium. The obtained suspension was centrifuged for 60 min at 10,000 rpm. The supernatant was separated and then filtered through 0.45 µm Millipore. The filtrate was diluted using 75% ethanol and measured specrophotometrically. The amount of free drug was determined in the filtrate and the amount of incorporated drug was determined as a result of the initial drug minus the free drug. The entrapment efficiency was calculated using the following equation.

$$\text{Entrapment efficiency} = \frac{W\ \text{initial drug} - W\ \text{free drug}}{W\ \text{initial drug}} \times 100$$

Where “W initial drug” is the mass of initial drug used and the “W free drug” is the mass of free drug measured in the supernatant after centrifugation of the aqueous dispersion.

5.6. Percentage yield:

The percentage yield is calculated to know about the efficiency of any method, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of nanoparticles recovered from each batch in relation to the sum of starting

material. The percentage yield of prepared nanoliposomes was determined by using the formula.

$$\text{Percentage yield} = \frac{\text{Practical yield}}{\text{Theortical yield}} \times 100$$

APPLICATIONS OF NANOLIPOSOMES

Nanoliposome in nanotherapy.

The bioactive materials need novel drug delivery technologies to reduce toxic effects (Hughes., 2005). The delivery of some bioactive materials to special sites in the body and their release behavior is directly influenced by particle size. Nanocarriers have the potential to increase solubility, enhance bioavailability, improve time-controlled release and facilitate precision targeting of the entrapped compounds to a greater extent due to more surface area (Mozafari, 2006). As a result of improved stability and targeting, the amount of materials required for a specific effect when encapsulated in, is much less than the amount required when unencapsulated. The targeted drug release improves the effectiveness of bioactive compounds. It has a wide application range and ensures optimal dosage, thus improving cost effectiveness of the product.

Oral delivery: The application of nanoliposomes as oral drug delivery system is complicated due to the poor stability of the vesicles under the physiological conditions. However, there are various studies and current publications that point out the potential of phospholipid-based nanoliposomes to improve the bioavailability of poorly soluble and low-bioavailability drugs (Gert Fricker et al., 2010). The Polymerized, microencapsulated, and polymer-coated nanoliposomes have increased in the potential of oral nanoliposomes. Using targeted liposomes and a better understanding of their cellular processing will eventually lead to effective therapies from oral liposomes (Rogers et al., 1998). The Cyclosporin A - lecithin vesicles with an incorporation of >98%, and its comparative study with the marketed CyA-formulation Sandimmun Neoral® in rabbits proved that the both formulations are equivalent after oral administration (Guo et al., 2001).

Transdermal delivery: Transdermal drug delivery system is one of the most powerful approaches to encapsulate drugs in liposome to enhance drug efficiency. The liposome-based drug delivery can essentially be applied to any drugs. However many liposomes have been formulated to deliver a variety of drugs into the body through diffusion across the skin layers. Although, application had been limited due to the barrier properties of the stratum corneum, the outermost layer of skin. The attention in designing transdermal drug delivery systems was relaunched after the discovery of elastic vesicles i.e. transferosomes and ethosomes. Liposomes having celecoxib were prepared and were evaluated; the results showed that the maximum drug encapsulation efficiency was 43.24%. Drug release profile showed that 81.25% of the drugs released in the first 24 hours of the experiment (Moghimipour et al., 2015).

Pulmonary delivery

Targeted drug delivery to the lungs is one of the most widely investigated systemic or local drug delivery approaches. This route also makes it possible to deposit drugs more site-specific at high concentrations within the diseased lung thus reducing the overall amount of drug required to patients as well as reducing side effects. The attention in designing transdermal delivery systems was the discovery of various elastic vesicles. An ethosomes formulation provides a number of important benefits including improving the drug's efficacy, enhancing patient compliance, comfort and reducing the total cost of treatment. The delivery of liposome-entrapped antioxidants via the tracheobronchial route has been found to be very useful in increasing the half-times of the administered agents, thus providing a sustained release effect for prolonged drug action. The entrapment in liposomes of α-tocopherol, an exceptionally insoluble but extremely effective antioxidant, has been shown to be very effective in ameliorating oxidant-induced injuries in the lung. The use of bifunctional liposomes containing two antioxidants have been determined to offer excellent resistance to an oxidative damage and improved their clinical applications in antioxidant therapy (Shek et al., 1994). Pulmonary lung targeting finds applications in drug delivery to the lung itself and to other body organs via blood circulation. Perceptive pulmonary drug delivery systems towards enhancing their efficacy need (Chattopadhyay., 2013).

Intravitreal applications

Liposomes represent the first injectable systems for intravitreal administrations. Liposomes can give sustained release drug profile. In addition, liposomal formulation can minimize the tissue toxicity and enhance the intravitreal half-life of drugs by declining rapid clearance from vitreous cavity (Alghadyan et al., 1998). A multivesicular liposomal (MVL) drug delivery system comprising of Acyclovir Sodium, which forms a depot on intramuscular injection. The MVL provides control release of acyclovir for extend period of time and is advantage over oral route as the absorption is dose dependent and extremely variable with a bioavailability varies from 10% to 30% (Jain et al., 2005). The fluconazole-encapsulated liposomes which were administered intravitreally in rabbit eyes. Entrapping of fluconazole into liposomes considerably slowed down clearance of free fluconazole after intravitreal injection and thus achieved higher fluconazole concentration in the vitreous (Gupta et al., 2000).

Cancer therapy

Liposomes are used as a carrier for drugs in the treatment of cancer and are beneficial because the liposomes promote passive targeting for the cancer cells. Unlike the blood vessel cells in healthy humans, tumor cells have an enhanced permeability and retention effect, allowing the passage of larger molecules. Drugs encapsulated with liposome up to the size of 400nm can enter tumor sites easily but are restricted from the healthy tissues by the endothelial wall. Thus, the drug molecules are targeted to the tumor cells other than healthy tissues in the body (Malam Y, et al., 2009).

6.7 Gene therapy

The nucleic acid drugs are in the early stages of clinical trials, they can be considered as promising therapeutic agents for treatment of diseases such as hereditary disorders, cancer, neurological and cardiovascular disorders, AIDS and other viral infections (Ulrich et al., 1999).

Cationic liposomes and nanoliposomes are the mainly used nonviral vectors that are oftenly applied in gene therapy (Audouy et al., 2002). The capacity to mediate transfection was credited to spontaneous electrostatic interaction between them and negatively charged DNA molecules that ensures an efficient condensation of the polynucleotides. Modification in the lipid composition causes an appropriate charge of liposome–polynucleotide complex to increase possibility of cellular uptake. The proposed mechanism of oligonucleotide uptakes from cationic liposomes may be either fusion or endocytosis (De Lima et al., 2001).

6.8. Multidrug resistance therapy

The multidrug resistance (MDR) is becoming a major barrier for chemotherapeutic treatment in the fight against malignant cancers. Because of the emergence of MDR, higher doses of chemotherapeutic drugs are needed which ultimately leads to intolerable toxicity and the death of patients. Multidrug resistance diminishes the efficacies of a broad range of chemotherapeutic agents; this leads the decrease of intracellular drug concentration and the failure of killing sufficient cancer cells. When co-administration of multiple chemotherapeutic agents cannot overcome MDR due to the different pharmacokinetic properties of combined drugs and only brings limited clinical advantage. Current development in nanomedicine and nanotechnology has enabled scientists to deliver multiple drugs of similar or different acting mechanisms into cancer cells with a predefined releasing profile. Therefore, combining nanotechnology and co-delivery technique has the great potential to selectively deliver multiple drugs to overcome MDR (Yuan Sun et al., 2016).

6.9. Application in Food Industry

On the basis of liposomal studies in the pharmaceutical and medical research it is applied in food industry. Researchers have utilized liposomes for the controlled delivery of functional components, such as peptides, enzymes, vitamins, and flavors in various food applications (Taylor et al., 2005). It is well known that encapsulated enzymes have improved stability and activity not only *in vivo*, but also *in vitro*. Liposomes isolate the encapsulated enzymes from the surrounding environment and providing them protection under conditions that would otherwise hamper the activity or even cause denaturation. Liposomes can also be used as a means of controlled release. Liposomes have also been used in dairy products to induce the slow digestion of lactose to aid the digestion of dairy products from the lactose intolerant (Matsuzaki et al., 1989). The bioactive compounds are essential in the design and production of functional foods, for improving the health status of consumers all around the world. Various clinical studies have established the beneficial role of eicosapentaenoic acid and docosahexaenoic acid in preventing diseases and reducing mortality from cardiovascular diseases. Oxidative deterioration of omega-3 fatty acids can cause the reduction in their nutritional quality. The liposomal formulations of these fatty acids could create a barrier against reaction with harmful environmental factors (Zahra Hadian., 2016).

6.10. Application in vaccines production

The vaccines produced by liposomal method is also known as virosomes (Wilschut J., 2009), that are constructed with viral surface antigens and synthetic lipids such as DOPC, DOPE or DPPC, which simulate viral membrane for vaccine delivery. When compared virosomes with conventional vaccines, virosomes exhibit the excellent immunogenicity as well as better biocompatibility and safety. These two liposomal vaccines, Epaxal and Inflexal V, have been permitted for clinical use (Usonis V et al., 2003). Epaxal is a hepatitis A virus (HAV) vaccine. Inflexal V is an influenza vaccine which has been used worldwide for fifteen years. In a clinical study involving 453 children, Inflexal V achieved a significantly higher seroprotection rate (88.8%) for H_3N_2 virus than that of a conventional influenza vaccine (78.3%), indicating the better immunogenicity (Kanra G et al., 2004).

6.11. Combination therapy with nanoliposomes.

The basic principle behind combination chemotherapy is the combination of drugs with different mechanisms of action and non-overlapping side effects these can be applied for the development of nanomedicines. However different types of combinations have been use in recent years, with at least additive effects in therapeutic outcomes for the combinations compared to individual therapies. Thus a number of different types of therapeutic combinations have been used (Mayer et al., 2006). Combinations involving one or two different liposomal drugs targeted against two or more different antigens on the same cells, or on two or more different types of cells (Theresa et al., 2013).

6.12. Clinical development of liposomes

Liposomes have now entered in the mainstream as sustained release drug delivery systems (Allen, Cullis., 2003). It is used for the in vivo delivery of all from small molecule therapeutics to nucleic acids. The long circulating (PEGylated) liposomes were shown to have widespread accumulation in Kaposi's sarcoma and head and neck cancers. Liposomes with intermediate accumulation in lung cancer and lower accumulation in breast cancer in an initial study using small numbers of patients (Allen and Cullis, 2013).

Table 3. List of approved liposomal formulations.

Drug	Product name	Type	Lipid composition	Route of administration	Approved treatment
Amphotericin B	Ambisome	Liposome	HSPC, DSPG & cholesterol	Intravenous	Sever fungal infections
Doxorubicin	Myocet	Liposome	EPC & cholesterol	Intravenous	Metastatic breast cancer
	Doxil	PEGylated liposome	HSPC, cholesterol & DSPE-PEG_{2000}	Intravenous	Kaposi's sarcoma, ovarian and breast cancer
Daunorubicin	DaunoXome	Liposome	DSPC & cholesterol	Intravenous	Blood cancer
Verteporfin	Visudyne	Liposome	EPG & DMPC	Intravenous	Age-related molecular degeneration
Cytarabine	Depocyt	Liposome	DOPC, DPPG, cholesterol & triolein	Spinal	Neoplastic meningitis and lymphomatous meningitis
Morphine Sulfate	DepoDur	Liposome	DOPC, DPPG, cholesterol & triolein	Epidural	Pain
Vincristine Sulfate	Marqibo	Liposome	Egg sphingomyelin and cholesterol	Intravenous	Acute lymphoblastic leukemia

Application in cosmetics

Now a day nanotechnology being used frequently in skin care goods. It is rapidly becoming a common in medicine and skin care products. It has poor reorganization what the technology, benefits, or possible implications of its use. The type of nanotechnology that is most significant in cosmetics, skin care and health products is the use of nanoparticles. Nanoliposomes are one of the most recognized technologies for the nanoparticles used in skin care and cosmetic products, and we are also well-known with the term liposome also. With this connection between the two is the possibly the best way to clarify what nanoliposomes are (Fakhravar et al., 2016).

CONCLUSION

Nanostructured delivery systems are promising candidates that permit to an efficient and targeted delivery of bioactive compounds. Lipid based carrier systems that include liposomes and nanoliposomes, are among the most promising encapsulation technologies used in the rapidly emerging field of nanotechnology. Nanoliposomes are one of the sole drug delivery system, which can be of potential use in controlling and targeting drug delivery. Liposomes can be administrated orally, parenterally and topically as well as used in cosmetic, sustained release formulations, diagnostic purpose and as good carriers in gene delivery various drugs with liposomal delivery systems have been approved. In current years, a number of important formulations for the treatment of different diseases have been developed. Liposomes permitted an important vascular carrier for therapeutic efficiency in term of duration of action and decrease in dose frequency and delivering drugs at higher efficacy and lower toxicity. Over all liposomal formulations are still important and successful accesses for the clinical application of nanomedicines. Therefore, it is sensible to project that this field will experience steady growth for the future prospective.

CONFLICT OF INTEREST

None declared.

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