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ISSN: 0975-7538
Review Article

Liposomes as novel drug delivery system: A comprehensive review

Nagasamy Venkatesh Dhandapani*[1], Anup Thapa[2], Goti Sandip[2], Ayush Shrestha[3], Niroj Shrestha[3], Rajan Sharma Bhattarai[4]

[1]Department of Pharmaceutics, [2]Department of Pharmaceutical Analysis, [3]Department of Pharmacology; JSS College of Pharmacy, Udhagamandalam – 643001, Tamil Nadu India
[4]Formulation, Research and Development, Dr. Reddy's Laboratories Private Limited, Hyderabad, India

ABSTRACT

Liposome is a microparticulate colloidal vesicle, in which aqueous medium is surrounded by single or multiple concentric layers of phospholipids. Both hydrophilic & hydrophobic drug can be incorporated, water soluble drug being trapped in aqueous core and fat soluble drug in phospholipids. It offers controlled release, targeted drug delivery thus enhanced therapeutic efficacy and reduced dosing frequency. Several liposome based drug formulation are approved for clinical use and many are under extensive investigation. Therapeutically, these are used as carrier for drugs, viruses, bacteria, antigen, peptides (antibiotic), vaccines, genes and diagnostic agents. This review discusses about the method of production and extensive therapeutic potential of liposomes as carriers for targeted and controlled delivery.

Keywords: Liposomes; Controlled release; Carrier; Drug targeting

INTRODUCTION

A liposome is a tiny bubble (vesicle), with a membrane composed of a phospholipid bilayer. Membranes are usually made of phospholipids like phosphatidyletlhanolamine and phosphatidylcholine. Phospholipids are amphiphilic with its polar head as hydrophilic and hydrocarbon tail as hydrophobic.

Advantages

1. They offer targeted drug delivery.
2. They are biocompatible, biodegradable and biologically inert.
3. They are nonantigenic, nonpyrogenic and non toxic.
4. They can encapsulate both water soluble and water insoluble drugs.
5. Drug toxicity is removed as other tissues and cells are protected.
6. Cellular uptake of drug is enhanced.
7. Size can be varied to incorporate smaller or larger drug molecules.

* Corresponding Author
Email: nagasamyvenkatesh@rediffmail.com
Contact: +91- 8903123467
Received on: 11-02-2013
Revised on: 25-04-2013
Accepted on: 27-04-2013

Disadvantages

1. Liposomes are less stable.
2. They are rapidly removed by cells of reticuloendothelial system (RES) from blood after iv injection.
3. Drug release is slow and influenced by phagocytes.

Methods of preparation of liposomes

Thin- film hydration method/Hand shaking method

This method was developed by Bangham et al, for the preparation of multilamellar vesicles. Briefly, phospholipids are dissolved in a mixture of organic solvents (chloroform and methanol). The lipids are deposited as stacks of film from the organic solvents on the wall of round bottom flask by the process of rotary evaporation under reduced pressure. Upon hydration of lipids by addition of aqueous buffer containing the drugs, lipids tend to swell and peel off from the walls of round bottom flask results in the formation of multilamellar vesicle. A mechanical energy is required to cause swelling of lipids and dispersion of lipids film by simple hand shaking technique. Alternatively, exposing lipid film into a water saturated nitrogen for a stipulated period of time usually 15 minutes also results in the swelling of lipids without the use of agitation.

The two critical factors needed to be noted here are, hydration time of lipids and the conditions of agitation that determine the entrapment of drug in the aqueous buffer in the internal compartment of MLV's. For an instance it was reported that more of the aqueous phase can be sequestered upon hydration of lipid over 20 hours with gentle shaking. This ultimately results in

slow hydration and greater entrapment of aqueous volume.

It was reported that the MLV's produced by hand shaking method and vesicles produced (LUV's) by non-shaken method exhibited a higher encapsulation efficiency (upto 30%). However, certain disadvantages in this method are the encapsulation and larger quantity of water soluble compunds are lost during the swelling and ultimately results in less entrapment of drug (10-15%). While, incase of lipid soluble component a 100% encapsulation efficiency is possible if the components are adequately present and without affecting the structural composition of the membrane (Bangham et al., 1965).

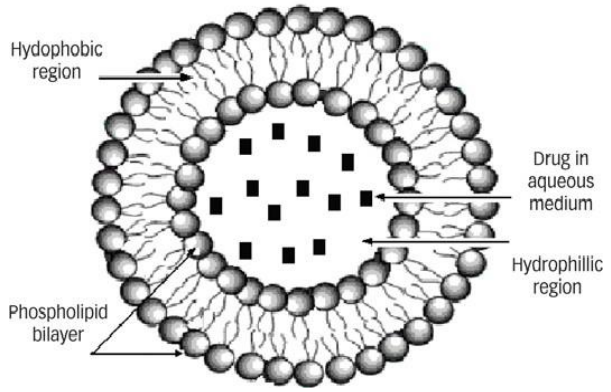

Figure 1: Diagramatic representation of Liposomes

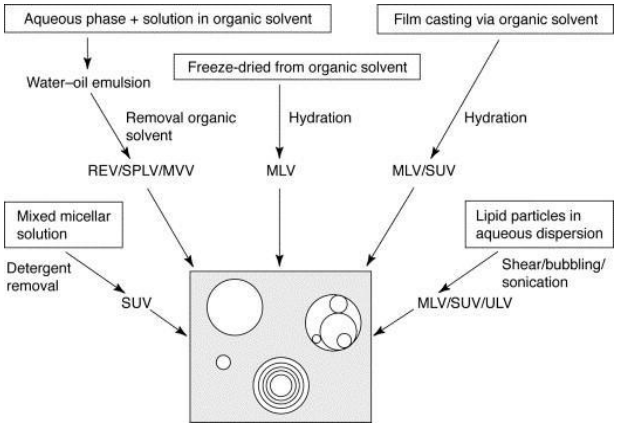

Figure 2: Method of preparation of liposomes by hand shaking method

Sonication method

This method is widely followed for the preparation of small unilamellar vesicles using bath type or probe sonicator in an inert atmosphere of nitrogen or argon. The basic principle of sonication involves the usage of high frequency sand waves in the suspension of MLV's causing the disruption, produces SUV's with a size range of 15-50 nm. The main purpose of sonication process is to produce a homogenous dispersion of small vesicles for greater tissue permeability. The most widely used sonication are bath and probe type. In probe type sonication, a high sonic energy is delivered to the liposome dispersion. The main disadvantage of this method is the over heating of lipid suspension leads to degradation of lipids. Also, the probe tip tends to release titanium partially into the liposomal suspension, which needs to be removed using centrifugation process. To overcome these problem related with probe sonicator, bath sonication method is more widely used.

By this method, a test tube containing the lipid suspension is placed in the bath sonicator and sonicating for 5-10 minutes whose the phase transition temperature of lipids (the temperature at which the lipid melts). Both the sonication methods are equiactive in the breakdown of MLV's into smaller structures. The results of size reduction of liposomes decreases the amount of water soluble drugs that can be entrapped. However, bath sonication process has the advantage over the probe soncation in term of preventing the degradation of lipids, shedding of metal from the titanium probe, generation of radioactive traces, chemicals and agents that could cause serious biohazards. Bath sonication process being a closed system that allows for temperature control which minimizes the thermo degradation of lipid and entrapped drug. Other factors like the position of the tube and the level of water in the bath also be for the reduction in the vesicle size.

However, few drawbacks are observed in the preparation of liposomes by sonication process are; the oxidation and hydrolysis of phospholipids and fatty acids. Another drawback is the denaturation of thermolabile substances (DNA and protein) to be entrapped. Liposome dispersion after sonication is placed in a clear plastic tube for high speed ultra centrifugation process for 100000 g 30 mon, 20^0C the sedimentation of titanium particles and multilamellar vesicles. After spinning the tube is carefully removed fron rotor and the top clear layer is decanted. This top layer usually constitutes the dispersion of small unilamellar vesicles with varying diameter in nano size range. Other factors like composition of lipid membrane, its concentration, temperature of hydration, sonication time, sonication volume and tuning also influencing the size and vesicle distribution (Lasic et al., 1988).

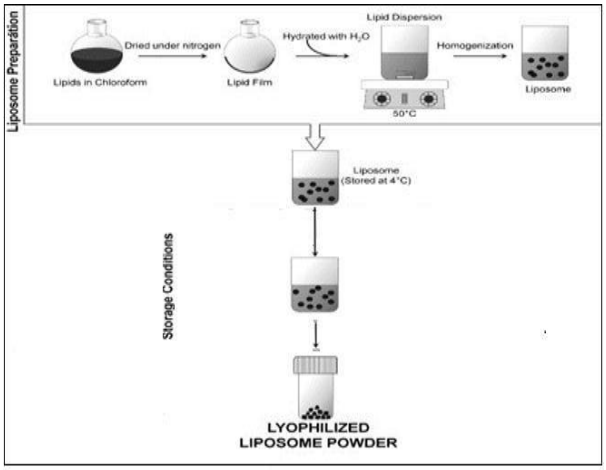

Figure 3: Preparation of liposomes by bath sonication process

Reverse phase evaporation method

Papahadjopoulos et al., developed the preparation of liposomes using reverse phase evaporation method. This process involves a rapid injection of aqueous solution containing the drug into an organic solvent containing lipid followed by bath sonication and mixture of two phases. The water droplets in the organic solvent form a water in oil emulsion. The emulsion is dried to obtain a semi solid gel in a rotary evaporator. Further, the gel is subjected to vigorous mechanical agitation using a vortex to induce a phase reversal and oil in water dispersión (an aqueous suspension of vesicles). During the mechanical agitation, the lipid monolayer encloses water droplets to form collapsed vesicle to form external phase, where as the remaining portion forms the entrapped aqueous portion. Upon conversion of gel to a homogenous free fluid, the dispersion is subjected to dialysis to remove the residual organic solvent. Large unilamellar vesicles costs an average diameter of (0.1-1 μm) are formed in the process. In this method, the encapsulation percentage is approximately found to be 50%. This method is widely employed to encapsulate macromolecules such as RNA, enzymes without losing their activity. However, there are certain limitations in this method, exposure of the material to be encapsulated to organic solvents and agitation can lead to denaturation of proteins and DNA strands (Cortesi et al., 1999).

High pressure extrusion method

This is an another method of producing SUV from MLV's. By this method, suspensions of MLV's prepared by film hydration method are repeatedly passed through polycarbonate membrane filters with small pore having diameter of 0.8 to 1 nm under high pressure (250 psi). By selecting appropriate filters, liposomes of desirable diameter can be produced. By forcing MLV's through small pores leads to the removal or peeling of successive layers and only one layer remains at the end. Apart from reduction of vesicular size, the extrusion method produces liposomes of uniform homogenous size distribution. Usually, low pressure ≤ 1 Mpa is employed when the lipid concentration low, but a pressure of 5Mpa is employed for the production of liposomes. This method is used for small scale production of liposomes, recently Schneider et al developed a new extrusion apparatus with an operating high pressure of 10Mpa with high output (Kleiner et al., 1989; Frezard et al., 1999).

Therapeutic application of liposomes

1] Drug targeting

An ideal targeted drug delivery delivers drug only to its site of action. Drug targeting leads to increased efficacy at low dose with decreased toxicity (Bangham et al., 1991). Methods to achieve active targeting via liposome involves use of ligands eg.cell specific antibodies, sugar residues, apoproteins or hormones etc which are tagged on lipid vesicle, these ligands recognises specific sites so cause targeting of liposomal drug at those target sites (Torchilin et al., 1985). Ligand is selected based on its recognition and specifically to target site. In cancer treatment, drug targeted to tumour cells via receptor specific ligandes, which may be specific antibodies for antigens produced by tumour cells.eg.MT_1-MMP (membrane type-1 matrix metalloproteins) plays important role in angiogenesis. In a study, anti MT_1-MMP antibobies were used as targeting ligand for doxorubicin (Hatakeyama et al., 2007). A novel liposomal formulation of paclitaxel targeting folate receptor was prepared which was designed to overcome vehicle toxicity associated with traditional cremophor ER based formulation (Wu et al., 2006). This formulation also provided prolonged systemic circulation time. The pharmacokinetics parameter of the meglumine antimoniate assessed in blood and organs of mononuclear phagocyte system showed approximately 3 fold improvement in targeting of reduced vesicles compared to its formulation in large sized liposomes (Dante et al., 2006). Paclitaxel liposomes shoved significantly higher targeting properties compared to paclitaxel injection for lung cáncer (Ling et al., 2011). Retention of cromolyn sodium was improved about 300 times in aerosolized liposomes compared to inhalers available in market (Gupta et al., 2006). In gene therapy, comodified liver targeting of cationic liposomes may deliver antisense oligonucleotides into hepatocytes infected with hepatitis B virus (Yuan Zhang et al., 2007). Stealth liposomes may target small inferring RNA(siRNA) to inflamed tissue though secretory phospholipase A_2 trigened release, to prevent cytokine expression in rheumatoid arthritis(Camilla et al., 2007).

RMP-7(ligand to B_2 receptor on brain microvacular endothelial cells) targeted liposomes may facilitate CNS targeting of therapeutic peptides & proteins (Ying Xie et al., 2005).

2] Topical drug delivery

Liposomes have shown great potential in dermatology and cosmetology (Schafer-Korting et al., 1989).When applied topically, liposomes exhibited an increased penetration of drug, thus enhanced permeability through skin but offered less side effects, because of reduced dose and limited systemic absorption (Lasch et al., 1986). In an experiment in guinea pigs, liposomal lidocaine was found to have higher concentration than its cream formulation (o/w) which proves enhanced penetration by liposome carrier system causing drug release in epidermis (Foldvari et al., 1990). Liposomes applied to skin in the form of solution or hydrogel where hydrophilic polymers are used as thickeners (Gabrijelcic et al., 1995). A study showed enhanced penetration in to skin by hydrogels prepared from Xanthngum (Gabrijelcic et al., 1990). Liposomal encapsulated drug of ketoconazole showed sustained release, increased antifungal efficacy and less adverse effects (Patel et al., 2009).

3] Antimicrobial therapy

Incorporation of antibiotics in liposomes offer two benefits:

1. Protection of drug eg.penicillins, cephalosporins etc against enzymatic degradation (eg. by β-lactamase)

2. Enhanced cellular uptake of antibiotic in microorganism, thus reducing effective dose and toxicity as in liposomal amphotericin (Gorin et al., 1990).

Meglumine antimoniate incorporated liposomes may provide better treatment against visceral leishmaniasis, allowing lower number of injection, compared to conventional treatment (Frezard et al., 2000).

4] Antiviral therapy

A study showed effectiveness of liposomes as earlier of antiretroviral agent dideoxycytidine-5-triphosphate (Oussore et al., 1999). Encapsulation of this antiretroviral agent into liposome reduces the effective dose which prevents the dose related toxicities associated with agents.

5] Protection against enzymatic degradation

Lipids in liposomes formulation not prone to enzymatic degradation, so entrapped drug is protected when lipid vesicle in circulation in extracellular fluid (Bangham et al., 1965). Inside the cell, entrapped drug gets released either by diffusion, or dissolution of shell or degradation by lysosomal enzymes. Liposomes protect drug in GI environment (Rowland et al., 1980) and facilitates GI transport of different type of compounds (Dapergolas et al., 1976). Thus, liposomes have great potential for delivery of insulin & proteins, which are orally biodegradable.

6] Immunotherapy

Liposomes are used as carrier of vaccine agents. They are now employed as oral vaccines in immunization procedure. They can elicit both humoral, cell mediated immunity and exhibits potential base for oral vaccines against hepatitis A. Multiple antigen peptides (MAP) enclosed liposome trigerred strong immune response, suggesting its role for design of therapeutic vaccines (Chen et al., 2005).

7] Local therapy

Antioxidants like calabase, SOD delivered via anionic liposomes may provide better, targeted treatment in chornic inflammation of colonic epithelium, like ulcerative colitis (Tareq et al., 2006).

8] Prophylaxis

Butylcholinesterase-encapsulating bioadhesive liposomes may provide prophylaxis against organophosphate poisoning, by preventing loss of intracellular enzyme activity (Sharon et al., 2005).

9] Liposome potentialities for intravitreal administration

Marketed liposome-based medicines are all given parenterally (Ambisome®, Doxil®, DaunoXome® Novasome® and Nyotran®) and only one named Visudyne® is employed for the treatment of age-related macular degeneration, however, by the intravenous route. liposomes are able to control drug release and improve significantly their vitreous half-life. Moreover, they might reduce the amount of drug that needs to be administered and consequently, the volume to be injected since large volume could result in an intraocular pressure increase as shown by many authors (Amrelie Bochot et al., 2012; Singh et al., 2004; Benz et al., 2006; Hollands et al., 2007).

Table 1: Advantages and drawbacks of liposomes for intravitreal administration

Advantages	Drawbacks
• Increase the stability of entrapped drugs • Reduce the drug toxicity • Possibility of ligands attachment • Reduce the number of administrations	• Possibility of aggregation during storage or *in vivo* when colloidal stability is poor • Blurring the vision • Cationic surface charge might induce Inflammation

10] Liposomes in ultrasonic drug and gene delivery

Investigations have found liposomes as promising drug delivery agents for mediating the accumulation of therapeutic agents at specific disease sites in the body (Torchilin et al., 2005; Andresen et al., 2005; Samad et al., 2007; Lian et al., 2001). Ultrasound-based approaches are now being developed for improving drug and gene delivery by increasing the transport of the therapeutic agent across the cell membrane or endothelial barriers (Mitragotri et al., 2005; Ter Haar et al., 2007; Duvshani-Eshet et al., 2007).

Echogenic liposomes, used to enhance ultrasound facilitated drug and gene delivery exhibits following characteristics:

a) High drug and gene loading properties.

b) High targeting properties as those containing entrapped therapeutic agents can be conjugated to antibodies and targeted to specific disease sites, allowing high local concentrations and low systemic toxicity.

c) Image-guided drug and gene delivery due to ultrasound reflectivity.

d) Permeability of cells and tissues to different sizes of molecules is increased by the cavitation caused by ultrasound-triggered destruction of gas bubbles and thus the transport of the drug or gene into cells and tissues is facilitated.

Table 2: Therapeutic applications of liposomes

Drug	Mode of administration	Use	Advantages
Tobramycin	Pulmonary delivery	Pseudomonas infection, aeruginosa	Targeted delivery
Salbutamol	Pulmonary delivery	Asthma	Targeted delivery
Cytarabin	Pulmonary delivery	Acute-leukemias	Targeted delivery
Benzocain	Transdermal	Ulcer on mucous surface with pain	Controlled release
Ketoconazole	Transdermal	Candida- albican's	Controlled release
Hydroxyzine	Transdermal	Urtecaria, allergic skin disorder	Controlled release
Idoxiuridine	Ocular delivery	Herpex- simplex, Keratitis	Targeted delivery
Doxorubicin	Oral delivery	Cancer	Targeted delivery

Table 3: List of marketed products

Marketed product	Drug used	Company	Target diseases
Fungizone®	Amphotericin-B	Bristol-squibb, Netherland	Fungal infections, Leishmaniasis
VENTUS™	Prostaglandin-E_1	The liposome company, USA	Systemic inflammatory Diseases
ALEC™	Dry protein free powder of DPPC-PG	Britannia Pharm, UK	Expanding lung diseases in babies
Topex-Br	Terbutaline sulphate	Ozone, USA	Asthma
Depocyt	Cytarabine	Skye Pharm, USA	Cancer therapy
Novasome®	Smallpox vaccine	Novavax, USA	Smallpox
Epaxal –Berna Vaccine	Inactivated hepatitis-A Virions	Swiss serum & vaccine institute, Switzerland	Hepatitis A
Doxil®	Doxorubicin Hcl	ALZA, USA	Refractory ovarian cáncer
Evacet™	Doxorubicin	The liposome company, USA	Metastatic breast cáncer
Autragen™	Tretinoin	Aronex Pharm, USA	Kaposi's sarcoma
Shigella Flexneri 2A Vaccine	Shigella flexneri 2A	Novavax, USA	Shigella Flexneri 2A infections
Nyotran™	Nystatin	Aronex Pharm, USA	Systemic fungal infections

e) Ability to provide controlled bolus release with a single high amplitude ultrasonic pulse, sustained release by a series of low amplitude pulses, or a combination of the two.

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