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PROLIPOSOME: NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT

Liposome are biologically inert microscopic vesicles in which aqueous compartment is enclosed by a lipid bilayer membrane. Liposomes are most promising and broadly applicable drug delivery system. But liposomes are associated with poor stability problem and this leads to be storage problem .To overcome the stability problem proliposomes were discovered by Payne et al. in 1986.Proliposomes are defined as dry, free flowing particles with a dispersed system that can immediately form a liposomal suspension when in contact with water. Proliposome are helped to improve the bioavailability. This paper reviews the method of preparation, merits, evaluation of Proliposomes and highlights its potential to be exploited for different routes of administration.

KEYWORDS: Liposome, Proliposomes, Carriers, Phospholipids and Cholesterol and multi-lamellar.

INTRODUCTION

Since the discovery of liposomes in 1965 by Bangham et.al, they continue to be the most Promising, broadly applicable, and highly researched of all the novel delivery systems.[1] Structurally they are composed of phospholipids which are biodegradable, nontoxic and devoid of any antigenic, pyrogenic or allergic reactions, and with careful selection, allows encapsulation of matter that is as small as the lithium ion up to macromolecules as large as genetic material of several hundred thousand Daltons.[2, 3] These properties of liposomes have been extensively investigated for drug delivery, drugs targeting, controlled release and increased solubility. However, liposomes are relatively unstable colloidal system manifested by physical and chemical instability . Physical instability is evidenced by vesicle aggregation

and fusion, which is associated with changes in vesicle size and loss of entrapped material. Chemical stability is of more importance as it is associated with phospholipids which form the backbone of the bilayer. It is of two types namely hydrolysis of the ester bonds linking the fatty acids to the glycerol backbone and peroxidation of unsaturated acyl chains (if present) which accelerates liposome breakdown and alters drug-release characteristics.[4,5] These factors influence the in vivo performance and storage behaviour of liposomes.[6] For liposomes to enter the market, they must be stable during the storage period, and remain intact before reaching their targeted tissues to produce action. Various approaches have been used to overcome these problems, some of which include, control of particle size and lamellarity, altering the lipid composition, lyophilisation, electrosteric stabilization etc4. One such approach which helped overcome the stability issue associated with liposome and led to the development of a new drug delivery system is the Proliposome (PL).Discovered by Payne et.al in 1986, Proliposomes (PLs) are dry, free-flowing granular products composed of drug and phospholipids which, upon addition of water, disperse to form a multi-lamellar liposomal suspension.[7] It is one of the most cost-effective and widely used methods for producing commercial liposome products. It is based upon the intrinsic property of hydrated membrane lipids to form vesicles on contact with water. Being available in dry powder form, they are easy to distribute, transfer, measure and store making it a versatile system. Liposomes can either be formed in vivo under the influence of physiological fluids or can be formed in vitro prior to administration using a suitable hydrating fluid. The liposomes formed on reconstitution are similar to conventional liposomes and more uniform in size.[8]

Advantages

1. High entrapment of hydrophilic material.
2. Therapeutic benefits of proliposomes include enhanced bioavailability.
3. Protection of drugs from degradation in the GIT
4. Reduced toxicity and taste masking.
5. Relatively cheap.
6. Convenient to prepare
7. The proliposomes used for targeted drug delivery and controlled drug release.[9]

Distinguish between liposomes and proliposomes

Liposomes are unilamellar or multilamellar spheroid structures composed of lipid molecules, often phospholipids. They show controlled release and increased solubility but have a tendency to aggregate fuse, susceptible to hydrolysis or oxidation.

Proliposomes an alternative forms to conventional liposomal formulation composed of water soluble porous powder as a carrier, phospholipids and drugs dissolved in organic solvent. Lipid and drug are coated onto a soluble carrier to form free-flowing granular material show controlled release, better stability, ease of handling and increased solubility.[10]

Components of proliposomes

1. Phospholipids: The bulk components of liposomal lipid membrane are phosphotindyl glycerides (phospholipids), amphipathic molecules that consist of a hydrophilic phosphate head group and hydrophilic fatty acid chains bridged together by a glycerol back bone. In early studies, egg phosphatidylcholine (egg PC, egg lecithin) was used and these phospholipids. Although exhibiting a single head group composition, contain various lipid species due to the presence mixed and varying acyl chain lengths. More recently highly purified lipids have been chemically synthesized consisting of saturated fatty acid species with same numbers of carbons. The fatty acid chain can vary between 8-24 carbons (C8-C24); among them thee mostly used in liposomal drug delivery are myristic (C14), palmitic (C16) and Stearic (C18). Aside from the fatty acid carbon length, the phosphate group can varied and include phosphatidylcholine (PC).Phosphotindlyethanolamine (PE), which are zwitter ion (charge balanced with positive charge on head group and negative charge on phosphate group), the negatively charged phosphatidly, serine, and glycerol inositol head group. Many of physicochemical properties of liposomes such as stability, permeability, phase behaviour and membrane order depend on the fatty acid chain length and saturation.[11]

2. Cholestrol: It is added to increase the stability of phospholipids molecule. Cholesterol incorporation in phospholipids membrane increases the separation between choline head group and eliminates the normal electrostatic hydrogen bonding interaction.[12]

3. Solvent: Organic solvents are used for preparing proliposomes usually a mixture of chloroform and alcohol (methanol, ethanol).Alcohol is added to stabilize chloroform to avoid the production of phosphogene gas. They are also used to for providing the softness for vesicle membrane.[13]

4. Water soluble carriers: The carrier's chosen should have high surface area and porosity so that the amount of carrier required can be easily adjusted to support the lipids. It also enables high surfactant to carrier mass ratio in the preparation of proliposomes. Further, being water soluble they allow rapid formation of liposomal dispersion on hydration and by controlling the size of porous powder, relatively narrow range of reconstituted liposomes can be obtained. Some of the carriers utilized include- maltodextrin, sorbitol, microcrystalline cellulose, magnesium aluminium Silicates, Mannitol etc.[14]

Method of preparation

a) Film deposition on carrier method.
b) Spray drying method.
c) Fluidised bed method.
d) Super critical anti-solvent method

These are described as

i. Film deposition on carrier method

This is the original method used by Payne et.al in the preparation of proliposome. For preparing proliposome special equipment as Buchi rotary evaporator 'R' with water cooled condenser coil and a stainless steel covered thermocouple connected to a digital thermometer, is required. The end of the glass solvent inlet tube is modified to a fine point, so that the solvent is introduced into the flask as a fine spray. As shown in Fig1.

Procedure

The solution of lipid and drug in volatile organic solvent is prepared and carrier powder is introduced into 100ml flask. The flask is then fitted into the evaporator and rotated slowly so that the powder tumbles gently off the walls to ensure good mixing and the solvent is evaporated. The flask is lowered into a water bath at 50-55°C when a good vacuum as developed (around 100 KPa). An aliquot of 5ml of lipid solution is introduced into the flask via the solvent inlet tube. The solvent is absorbed completely by the powder and the temperature of the bed is monitored. As evaporation proceeds, the temperature will decrease. A second aliquot is introduced slowly when the temperature begin again. The temperature allowed rising to 300C, the vacuum is released and drying process is completed by connecting the flask containing the powder to lyophilize and leaving it evacuated overnight at room temperature.

For use, introduce 10ml of distilled water into one vial and mix on a whirl mixer for 30sec. or in shaking water bath above the lipid phase transition temperature, to give a 5%w/v solution of sorbitol (isotonic with normal saline) and a lipid concentration of 10mg/ml.[12]

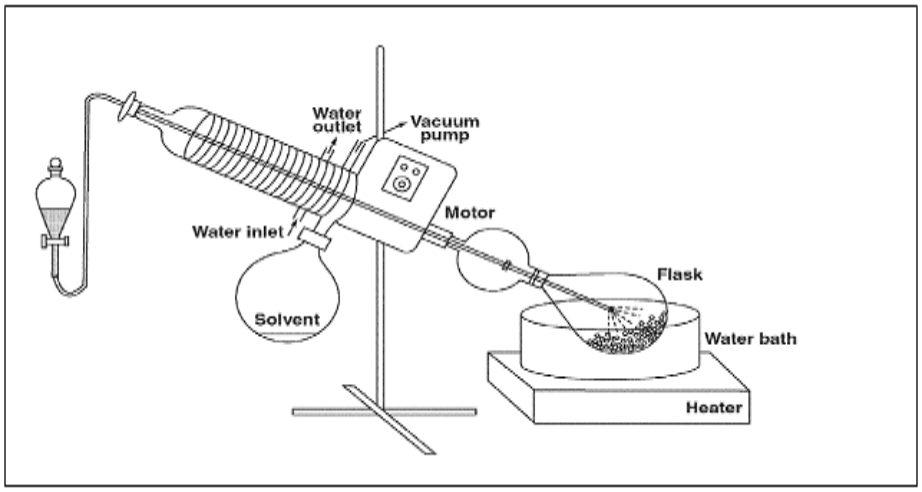

Figure-1: **Apparatus for preparing PLs by Film Deposition on carrier method**[15]

ii. Spray drying method: This method is mainly used when particles of uniform size and shape are required and can be easily scaled up it is cost effective and suitable for large scale production of proliposomes. The unique feature of spray drying process lies in its ability to involve both particle formation and drying in a continuous single step, allowing better control of particle. Spray drying is not only limited to aqueous solutions, but can also be used for non-aqueous systems to prepare particles.

The spray drying process involves four stages

1. Atomization of the product into a spray nozzle.
2. Spray-air contact.
3. Drying of the spray droplets, and
4. Collection of the solid product.

Procedure: Initially liquid dispersions containing pure lipid or lipids and carrier in organic solvent are prepared and pumped into the drying chamber. Shown in Fig2. The dispersions are atomized into the drying chamber using a spray nozzle and are dried in a concurrent air flow which is then collected in a reservoir. Major concerns to spray drying are high working temperatures, shearing stresses and absorption phenomenon that may lead to thermal and mechanical degradation of the active molecules. This can be improved by optimising the operating parameters such as drying air temperature and liquid spraying rate. Stabilising

adjutants such as disaccharides, cyclic oligosaccharides and polyols can also be used to protect the integrity of the active molecules and enhance the efficiency of hydration by increasing the surface area of lipids.[16]

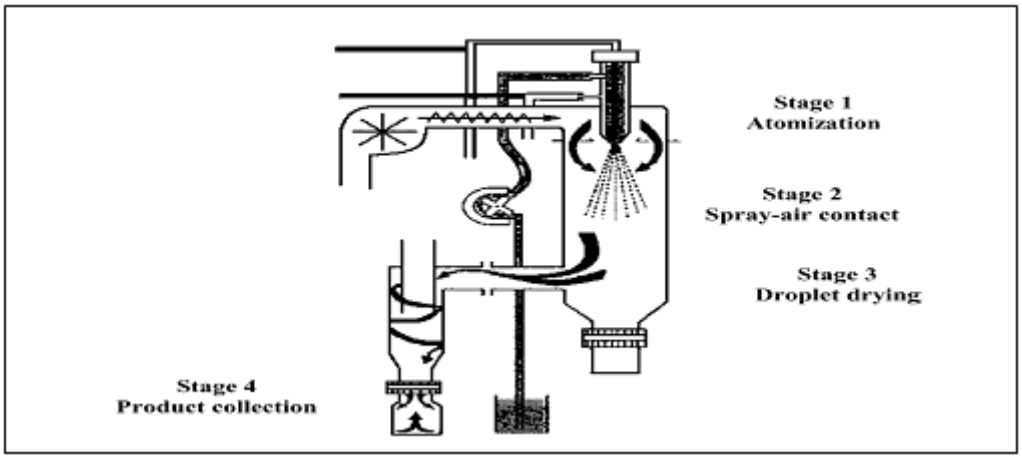

Figure-2: ***Apparatus for preparing PLs by Spray drying method.***[17]

iii.Fluidized bed method

Principle

It works on the particle coating technology. This method can be used for large scale production of proliposomes.

Method: The carrier material used here can vary from crystalline powder to non pareil beads. When using beads as carrier material, initial seal coating is applied to the beads to provide a smooth surface for further coating of phospholipids. This ensures formation of thin uniform coating of phospholipids around the core and formation of smaller sized liposomes upon hydration. Solution of drug and phospholipids in organic solvent is sprayed onto the carrier material through a nozzle. At the same time, the organic solvent is removed by applying vacuum to the fluid bed. To remove the trace amount of residual solvent the finished lipid-coated powder/beads can be dried under vacuum overnight.

Advantages of this method

a) It utilizes Film coating technology which is well established and process able.

b) Various cores and coating materials are available or easy to prepare.

c) It is a cost-effective method to prepare liposomes for drug delivery.[18]

iv. Super Critical Anti-Solvent Method

Supercritical anti solvent method utilises Supercritical Carbon dioxide (SCCO2) in the preparation of PLs.SCCO2 is a fluid state of carbon dioxide where it is held at or above its critical temperature and pressure. Anti-solvent technology is widely used in food industry and was developed to prepare PLs because of its lower residual solvents, simpler steps and mild operation temperatures. As shown in Fig.3, the apparatus used in the preparation of PLs include three parts: a sample delivery unit, a precipitation unit and a separation unit. The sample delivery unit consists of two pumps: one for CO2 and the other for solution. CO2 is supplied from the CO2 cylinder[1] which is cooled down by a refrigerator[2] and introduced via a high pressure pump[3] to the buffer tank,[4] in which it is preheated. The drug solution is introduced via HPLC pump.[11] The solvent used for dissolving the drug should be completely miscible with CO2. Opening the valves A and B allows the entry of solution and CO2 into the vessel through the nozzle (B). As seen in Fig 3B, solution is sprayed through the inner tubule whereas CO2 is sprayed through the outer tubule of the nozzle. The precipitation unit consists of a vessel.[9] heated by an air bath. The separation unit consists of a separator[13] and a wet gas meter.[14] The organic solvent is separated from SCCO2 in the separator because of lower pressure whereas volumetric flow rate of CO2 is measured by the wet gas meter.[19]

Evaluation

Proliposomes are evaluated by following parameters.

i. Scanning Electron Microscopy (SEM)

SEM is mainly used to view the surface morphology of the PL powder. This involves comparing the image of the pure carrier material with that of the PL. The image of the carrier material in the formulation confirms the deposition of phospholipids on the carrier and thus confirming the formulation of proliposomes.[20]

ii. Transmission Electron Microscopy (TEM)

TEM is mainly used to study the morphology of the liposomes formed after hydration of the PL powder. The process involves hydrating PLs with purified water and observing the shape and lamellarity of the liposome vesicles formed under the microscope.[8, 20]

iii. Hydration study

It helps to evaluate the ability of proliposomes to form liposomes on hydration. In this study a small amount of PL powder is placed on glass slide or on cavity slide and slowly adding

water drop wise while observing it under the microscope to view the formation of vesicles. During the hydration process dissolution/disintegration occurs.[8,20]

iv. Flow Property

A solid powder based formulation it is important to evaluate the flow property of PL. It also ensures that in spite of the deposition of phospholipids on carriers, the flowability of particles is not affected. Flow property is assessed by measuring the parameters such as Angle of Repose, Carr's Compressibility Index and Hausner's Ratio.[8,20]

v. Zeta potential

The zeta Potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. This can be used to study the surface charge of the particles.[20]

vi. Vesicle Size determination

Measurement of vesicle can be done by hydrating the PL powder followed by manual agitation and determining particle size using suitable Particle size analyser.

vii. Number of vesicles per cubic mm

Distinctive advantage of PL formulation is speculated only when abundant numbers of vesicles are derived from hydration of PLs which form an important prerequisite for optimizing the composition of the same. This is done by counting the liposomes formed after hydration under optical microscope using haemocytometer.[8]

Total no. of liposomes per mm3 is given by

$$\frac{\text{Total number of liposomes counted X Dilution factor X 4000}}{\text{Total number of Squares counted}}$$

viii. Entrapment Efficiency

Entrapment efficiency is determined by hydrating the PLs to form liposome dispersion followed by separation of unentrapped drug and determining the amount of drug entrapped. Unentrapped drug can be separated by ultracentrifugation[20] and ultra filtration.[8,12] Percent drug entrapment is given by,

$$\frac{\text{Entrapped Drug X 100}}{\text{Total Amount of Drug added}}$$

Applications

Proliposomes can be exploited for the following routes of administration:

Oral delivery

Oral drug delivery continues to be the preferred route of administration, but liposomes have limited success in delivering drugs through oral route. This is due to the absence of a stable dosage form oral delivery and erratic and unpredictable absorption profiles shown by liposomes. Being available as free flowing powder, PL represents the first example of delivering liposomes into solid dosage form such as tablets or capsules. Further, liposomes are formed on contact with biological fluids at the site of absorption ensuring the retention of liposome integrity. Zaleplon is a hypnotic drug indicated in insomnia and is a potential anticonvulsant. Due to its limited aqueous solubility and extensive first pass metabolism it shows poor bioavailability of 30%. PLs for oral delivery of Zaleplon and found 2-5 fold Improvement in oral bioavailability in rats compared to pure drug.[21]

Arthritis

Drug that are being in arthritis especially steroids, are destroyed by their peripheral effect. On local administration into the joints, drug diffuses easily from the site of injection and its action on the inflamed area is only transient. Segal et.al suggested that liposomes could be used in the treatment of local diseases. It is observed that steroids (e.g. cortisol palmitate) can be entrapped into large multilamellar liposomes composed of dipalmitoyl phosphatidly choline and phosphatidic acid. These preparations, when ingested into rabbits with experimental arthritis, can decrease the temperature as well as the size of the joints to a greater extent than with a similar amount of free steroids.[12]

Diabetes

The feasibility of using liposomes as a potential delivery system of the oral delivery of insulin has been extensively studied. Alteration in blood glucose level in diabetic animals was obtained by the oral administration of liposome encapsulated insulin. Dobre et.al demonstrated a lowering of blood glucose level in normal rats following the oral administration of insulin entrapped in PC: CH liposomes.[12]

Parenteral delivery

PLs are well suited for parentral application of liposomes. The main advantage associated with PLs is that it allows sterilization without affecting the intrinsic characteristics. Besides,

they can be stored as sterilized in dry state and can be hydrated prior to administration to form multilamellar liposomal suspension.[22]

Pulmonary Delivery

Major advantage of liposomes as pulmonary drug delivery system is that they are prepared from phospholipids which are endogenous to lungs as component of lung surfactant. Drug encapsulation in liposomes provides modulated absorption, resulting in localized drug action in the respiratory tract and prolonged drug presence in circulation and reduced systemic adverse effects.[23, 24] Drug delivery to the pulmonary route is achieved by three types of devices namely.

Pressurised metered dose Inhalers (pMDI)

As the name suggests it consists of solution or suspension of drugs in liquefied propellants. Use of Hydrofluroalkanes as non-ozone depleting propellants over CFCs has the limitation for liposome delivery as they are poor solvents for phospholipids. Proliposomes help overcome this limitation as they can be suspended in these propellants and serve as carrier for pulmonary delivery of liposomes through pMDI.[23]

Dry Powder Inhalers (DPIs)

These disperse the drug into the patient's airstream as a fine powder during inhalation. Delivering liposomes through DPI have many advantages such as controlled delivery, increased potency, and reduced toxicity, uniform deposition of drugs locally, patient compliance, stability and high dose carrying capacity. Being available as dry powder form, PLs are the best alternative for delivering liposomes through DPIs. Chougule et.al developed spray dried liposome encapsulated Dapsone DPI for prolonged drug retention in lungs to prevent Pneumocystis carinii pneumonia. Prolonged drug release of up to 16 h was observed in vitro.[23, 25]

Nebulizers

Nebulisation offers the simplest means, for delivering liposomes to the human respiratory tract but it is concerned with liposome leakage and drug stability. Use of dry powder formulations has been suggested to overcome these issues. Lyophilisation and jet milling may be used to obtain dry powder but tend to have deleterious effect on liposomes due to the stresses involved in these processes. Thus, PLs serve as a stable alternative for delivering liposomes through nebulisation. Besides, the ready formation of an isotonic liposome

formulation in situ from PLs seems to offer advantages over other formulation approaches.[23, 24]

Mucosal delivery

PLs form vesicular structures (liposomes) *in vivo*, triggered by the aqueous environment found on the mucosal surfaces. Phospholipids present in them have natural affinity for biological membranes. Besides they are generally nontoxic and non-irritant. The presence of drug as molecular dispersion in the bilayer offers improved drug activity. Further, the difficulties associated with liposomal preparations such as stability and loading are circumvented because the PLs convert to vesicular structures *in vivo*, i.e., on the mucosa.

Vaginal delivery systems are frequently required to treat local fungal infections. The poor aqueous solubility of antifungal and steroid compounds in conventional formulations limits their presence as molecular dispersion and consequently affects the drug concentration at active sites. The associations of these lipophilic agents with the phospholipids molecules of proliposome make them excellent carriers to molecularly disperse the drug. Nasal mucoadhesive delivery has been used to improve local and systemic delivery of therapeutic compounds. Limitations associated with this route are mucociliary clearance which limits the residence time of drug in the nasal cavity and lack of sustained release of drugs with short half-life. Proliposomal delivery helps to overcome these limitations. Liposomes formed on hydration decrease the mucociliary clearance of drug due to their surface viscosity and provide intimate and prolonged contact between the drug and mucus membrane.[26,27]

Transdermal delivery

Phospholipids, being the major component of liposomal system, can easily get integrated with the skin lipids and maintain the desired hydration conditions to improve drug permeation. When PLs are applied to mucosal membrane, they are expected to form liposomes on contact with mucosal fluids whereby the resulting liposomes act as sustained release dosage form for loaded drugs. Liposomes formed on hydration have the ability to modulate diffusion across the skin. They do so by fusing with the skin surface and establishing concentration gradient of the intercalated drug across the skin. Thus they enhance skin permeation. Also, the vesicle intercalation into the intracellular lipid layers of the skin results in fluidization and disorganization of the regular skin structure, obviating the barrier function of the stratum corneum.[22]

Ophthalmic delivery of drugs

The potential of liposomes in topical ocular drug delivery was first focused by Smolin et al. Schaeffer and Krohn and Schaeffer et al. liposomes offer advantages over most ophthalmic preparation in being completely biodegradable and relatively nontoxic. Smolin et al. reported the treatment of acute and chronic herpetic keratitis in albino rates, idoxuridine entrapped in liposomes was more effective than a comparable therapeutic regimen of unentrapped drug. Schaeffer et al. reported that transcorneal flux of penicillin G, indoxol and Carbachol were approximately double when these drugs were presented to the corneal surface in liposomal form. In direct contrast to these findings, Starford et al., observed a reduction in the fraction of epinephrine and insulin absorbed into aqueous humor in liposomes.[12]

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