

A REVIEW: PROLIPOSOMES AS A STABLE NOVEL DRUG DELIVERY

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ABSTRACT

Liposome, the microscopic phospholipids vesicles, and a novel drug delivery system that allow drug at the pre-established rate decided according to requirements, pharmacological aspects, drug profile, physiological conditions of body etc. But the liposome betrays low stability problem and due to this storage problem arise. To overwhelm this problem, Proliposomes (PLs) were discovered in 1986. Proliposomes are free-flowing granular products that consist of drug and phospholipids precursors which on hydration converted to the liposome. This paper reviews various features regarding pro-liposome, their method of preparation, evaluation, applications and focus its potential to be chosen for different routes of administration.

KEYWORDS: Liposome; Pro-liposome; Carriers; Phospholipids; Cholesterol.

INTRODUCTION

The liposomes were first developed by British hematologist Dr. Alec D Bingham in 1961 at the Babraham Institute, in Cambridge. The liposome word is derived from the Greek words: "Lipos" meaning fat and "Soma" meaning body.^[1] Out of all the novel drug delivery systems, liposomes are considered to be the most efficient systems.^[2] A micro-spherical vesicle

consisting of an aqueous core enclosed in phospholipids molecules is known as a liposome. Drug molecules can be incorporated into the aqueous phase or within the lipid bilayer.

They are preferably used as a vehicle for administration of nutrients and pharmaceutical drugs to improve the stability and effectiveness of the drug by reducing the side effects.^[3] To gain the place in the market, Liposomes should be stable and intact during the storage period and before reaching the targeted site to produce therapeutic action. However, because of physical and chemical instability, liposomes are relatively unstable colloidal systems.^[4]

Liposomal suspension may show limited shelf life and to overwhelm the stability issue related to the liposome, a new “pro-liposome” formulation is developed that can produce liposomes quickly when there is a need and without excessive manipulation.^[5] Pro-liposomes (PLs) were initially discovered in 1986.^[6] Pro-liposomes are dry, free-flowing granular products that on hydration or on contact with biological fluids in the body form the liposomal dispersion. They mainly consist of water-soluble porous powder and phospholipids.^[7] As they are in dry powder form, it's easy to distribute, transfer, measure, and store, making it a diverse system. Liposomes can either be formed by the influence of biological fluids in the body in vivo or in vitro using a sfavourable hydrating fluid before the administration from proliposomes.^[8] bioavailability and Solubility related problems of some drugs can be overcome by developing pro-liposomal formulations.^[9]

Advantages^[10]

- It can Target the drugs to non- reticuloendothelial tissues, which has not been possible with conventional liposomes.
- Proliposomes are consider as a control release drug delivery within the vasculature by manipulating the phospholipids composition of bi-layers.
- For the vascular originated disease
- Proliposomes have the best therapeutic effect over the conventional drug delivery systems.

Comparisons Between Proliposomes And Liposomes^[11]

Liposomes are unilamellar or multilamellar spherical shaped particulate structures consist of lipid molecules, often phospholipids. They show controlled release and enhance solubility but have a tendency to aggregate or fuse, susceptible to hydrolysis or oxidation. Proliposomes are alternative forms to conventional liposomal formulation having 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 enhanced solubility.

Components Used In The Preparation of Proliposomes

(1) Water-soluble carriers^[12]

The carriers chosen should have a 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, the relatively narrow range of reconstituted liposomes can be obtained. Some of the carriers utilized include- maltodextrin, sorbitol, microcrystalline cellulose, magnesium aluminum silicates, Mannitol etc.

(2) Phospholipids^[13,14]

The bulk components of liposomal lipid membrane are phosphatidyl glycerides (phospholipids), amphipathic molecules that consist of a hydrophilic phosphate head group and hydrophilic fatty acid chains bridged together by a glycerol backbone. 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 of 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, the 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 be varied and include phosphatidylcholine (PC). Phosphatidylethanolamine (PE), which are zwitterion (charge balanced with the positive charge on a head group and negative charge on phosphate group), the negatively charged phosphatidyl, serine, glycerol inositol head group. Many of physicochemical properties of liposomes such as stability, permeability, phase behavior, and membrane order depend on the fatty acid chain length and saturation.

(3) Steroids^[13,14]

Cholesterol can be consider as a commonly used steroid in the development of liposomes to reduces the permeability of water-soluble molecules through the membrane and to improve the stability of bilayer membrane in the presence of biological/physiological fluids.

Liposomes formulation without cholesterol may interact with the blood proteins like albumin, and macroglobulin, which results in destabilization of the liposomes. Cholesterol may help to reduce these interactions with blood proteins.

(4) Solvents

They are used to for give softness to the vesicular membrane. Examples of solvents are methanol and chloroform.

Methods For Preparation of Sln

- Film-deposition on carrier method.
- Spray drying method.
- Fluidized-bed method.
- Supercritical anti-solvent method.

1) Film deposition on carrier method

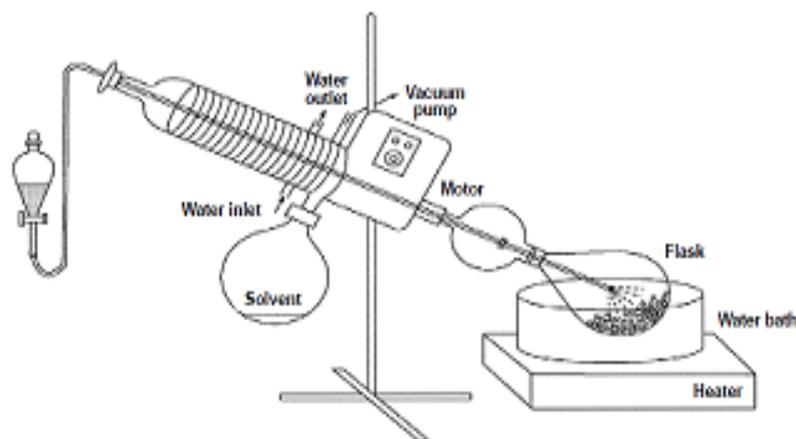


Figure: 1 Film deposition method for preparation of proliposomes.

Film deposition on the carrier is used for the formulation of Pro-liposomes. In this method, the coating of drug and phospholipids is discharged on a previous, water-soluble carrier substance. By seeing Figure 1, an evaporative solution which consist a solution of drug and phospholipids is injected drop by drop by a feed tube onto a core of carrier which is carried in a vessel of a rotary flash evaporator under vacuum. At any stated moment, the matrix's over wetting is circumvented and following an aliquot of the organic mixture is feeding solely when a free-flowing powder matrix is procured.^[15] The chosen carriers should exhibit a great permeability and surface area with respect to regulate the quantity of carrier which is needed to assist the lipids. As they are water soluble in nature, they are enable to fast production of

liposomal dispersion on hydration and by properly managing the size of previous powder, a comparatively limited variety of reconstituted liposomes can be acquired. Mostly used carriers are maltodextrin, sorbitol, microcrystalline cellulose, magnesium aluminum silicates, mannitol, etc.^[16] The stride of solvent inclusion and evaporation which is sluggish.^[17] To circumvent this issue, after the procedure by dispersing the carrier substance in an organic mixture of drug and phospholipids in a vessel of a rotary evaporator and then directing it to vaccum evaporation. By doing so, highly consistent and well-organized lipid distribution is achieved and a steady and less time taking procedure is gained in contrast to the actual procedure.^[18]

2) Spray drying method

The distinctive attribute of this process is reclined in its propensity to include particle composition and drying together in a consistent stride, permitting more desirable production of particles. This method can be used for any of the aqueous or non-aqueous systems for particles production. Predominantly, this process is utilized when invariable sized and shaped particles are needed and can be simply scaled up. Its price is effectual and acceptable for massive preparation of PLs.^[19-20]

As shown in Figure 2, this spray drying procedure consists of four phases: the atomization of the product into a spray nozzle, spray-air association, drying of the spray droplets and collection of the solid product.^[21]

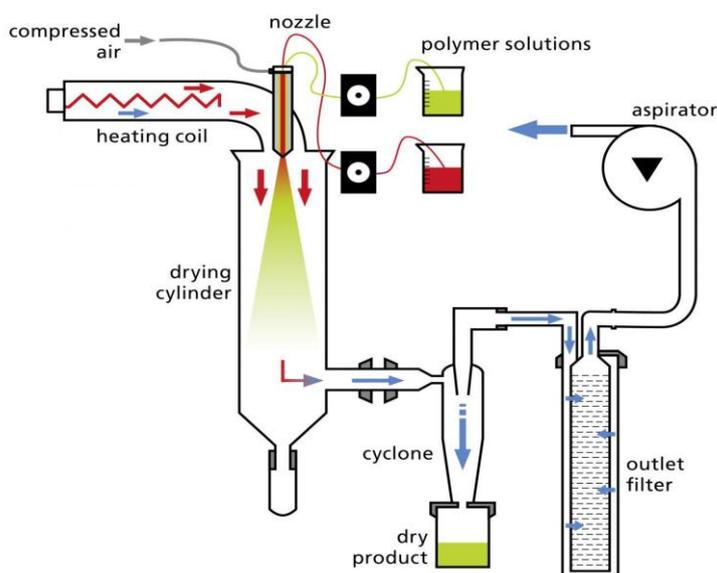


Figure: 2 Preparation of proliposomes by spray drying method.

Firstly, preparation of liquid dispersions carrying pure lipid or lipids and carriers in the organic mixture is done and then it is poured into the dry cell. By utilizing a spray nozzle, dispersions are atomized into drying cell and desiccated in a simultaneous air flow which is then gathered in a tank.^[21] Prime factors which affecting this method are high temperatures, shearing stresses, and absorption episodes and these can result in the thermal and mechanical degradation of active molecules. It can be upgraded by making the working variables better. Examples of working variables are drying air temperatures and liquid spraying rate. For shielding the unification of active molecules, stabilizing adjutants e.g. disaccharides, cyclic oligosaccharides and polyols can be utilized and by augmenting the surface area of lipids, the effectiveness of hydration can be intensifying.^[19-20]

3) Supercritical anti-solvent method

In a Supercritical anti-solvent method for the production of PLs, we use Supercritical Carbon dioxide (SCCO₂) which actually is carbon dioxide's fluid state when it is held at on some level above its critical temperature and pressure. Because of three following important factors:

- Lower residual solvents,
- Simple steps,
- Mild operation temperatures.

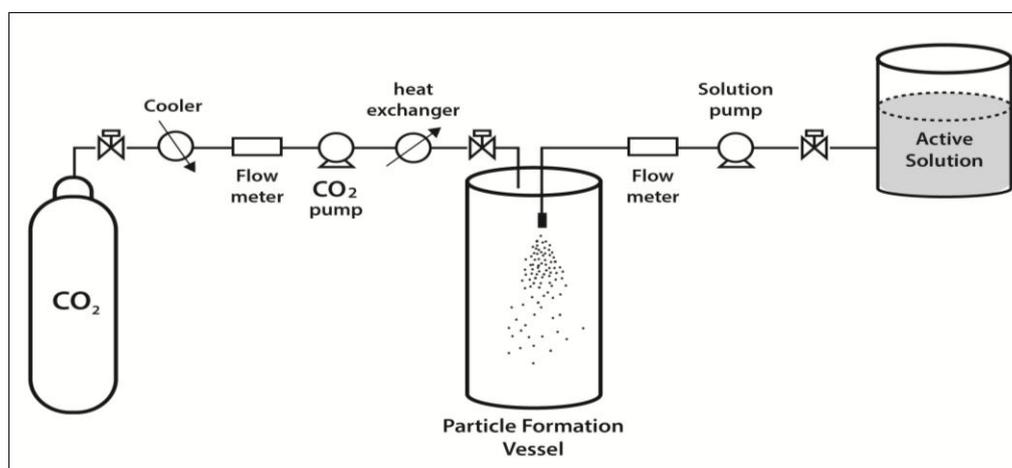


Figure: 3 Supercritical anti-solvent method.

The use anti-solvent technology for the preparation of proliposomes. As shown in figure 3 apparatus consisting of 3 parts (e.g. a sample delivery unit, a precipitation unit, and a separation unit) is basically used in those simple steps. Two pumps, one for the delivery of CO₂ which is supplied through CO₂ cylinder (72 cm³) after being cooled down by

refrigerator and a high-pressure pump is used introduce it to the buffer tank (-7°C) for preheating thus The conditions of temperature and pressure of the reaction vessel or CO₂ cylinder should be 45°C and 10 MPa and one for the drug solutions which is introduced via HPLC pump combines up to make the sample delivery unit.^[22] The solvent which is completely miscible with CO₂ should be used for dissolving the drugs. For both the preparations of proliposomes, phospholipids, cholesterol, and drug were dissolved in organic solvents followed by sonication until a clear and homogeneous solution is observed. For the entrance of carbon dioxide into the vessel by nozzle valves, A and B will be opened. Carbon dioxide is sprayed through the outer tubule whereas the solution is sprayed through inner tubule of the nozzle. The 2nd portion of the apparatus consists of heated by air bath vessel, and the last part comprises wet gas meter and a separator.

SCCO₂ is separated from organic solvent in the last part's separator because of its low pressure and on the other hand, a wet gas meter is used to measure the CO₂.^[23] After reaching the preset value of temp and pressure, valve A is open for the entrance of CO₂ right after that, valve B allow drug solution to enter the nozzle. The solution is mixed with SCCO₂ and diffused into each other rapidly like it is sprayed through the coaxial nozzle. Thus the solute will dissolve in an organic solvent to reach supersaturation in a short period of time about 30 minutes and this all because of the solubility of the solute in the organic solvent decreases gently, thus the proliposomes are precipitated in the vessel. After the complete utilization of solution, A and B valves are closed and valve C is opened to depressurize the vessel at the opening temperature and in the end, we collect these samples at the bottom of the vessel on the filter. The pressure, temp and the flow rate of the drug solution need to be optimized to obtain the high drug loading PLs.^[24]

4) Fluidized bed method

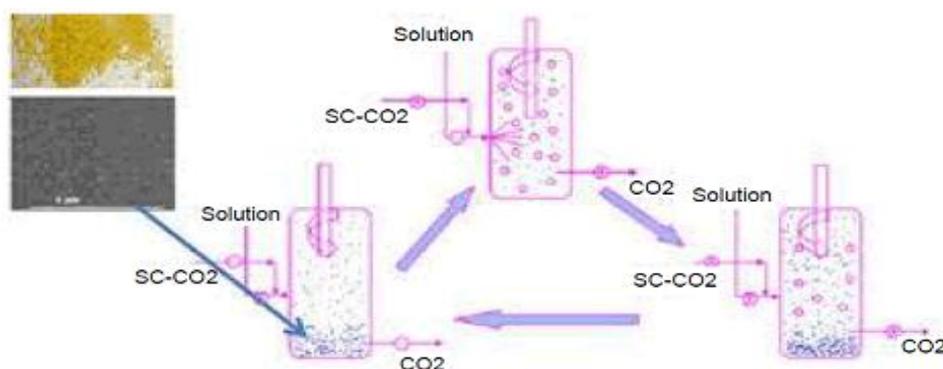


Figure: 4 Preparation of Proliposomes by using Fluidized bed method.

On the large scale production of proliposomes whose principle relays on particle coating technology, in which carrier material can vary from crystalline powder to non-pareil beads. While using non-pareil beads as carrier material, first for getting smooth surface pareil beads are coated with seal coating which can help further in a coating of phospholipids and which also ensure thin uniform coating formation of phospholipids around the core and small-sized liposomes upon hydration. Carrier material is then sprayed with the solution of organic solvent and solution of drugs through the nozzle, and by applying vacuum at the same time to the fluid bed organic solvent is removed. The trace amount of residual solvent is removed by the finished lipid-coated powder/beads when dried under vacuum overnight (Figure 4).

APPLICATION

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 the 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 a free-flowing powder, PL represents the first example of delivering liposomes into a 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.^[25]

Arthritis

The drug that is being in arthritis especially steroids is destroyed by their peripheral effect. On local administration into the joints, the 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 dipalmitoylphosphatidylcholine 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.^[10]

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 liposomes¹. Parenteral delivery PLs are well suited for parenteral application of liposomes. The main advantage associated with PLs is that it allows sterilization without affecting the intrinsic characteristics.^[16] Besides, they can be stored as sterilized in the dry state and can be hydrated prior to administration to form multilamellar liposomal suspension.^[27]

Pulmonary Delivery

The major advantage of liposomes as a pulmonary drug delivery system is that they are prepared from phospholipids which are endogenous to lungs as the 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.^[28-29] Drug delivery to the pulmonary route is achieved by three types of devices namely

(1) Pressurised metered dose Inhalers (pMDI)

As the name suggests it consists of solution or suspension of drugs in liquefied propellants. Use of Hydrofluoroalkanes 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 the carrier for pulmonary delivery of liposomes through pMDI.^[28]

(2) 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 the lungs to prevent *Pneumocystis carinii* pneumonia. Prolonged drug release of up to 16 h was observed in vitro.^[28,30]

(3) Nebulizers

Nebulization 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 a deleterious effect on liposomes due to the stresses involved in these processes. Thus, PLs serve as a stable alternative for delivering liposomes through nebulization. Besides, the ready formation of an isotonic liposome formulation *in situ* from PLs seems to offer advantages over other formulation approaches.^[28-29]

Mucosal delivery

PLs, form vesicular structures (liposomes) *in vivo*, triggered by the aqueous environment found on the mucosal surfaces. Phospholipids present in them have a natural affinity for biological membranes. Besides they are generally non-toxic and non-irritant. The presence of drug as a molecular dispersion in the bilayers 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.^[31] 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 phospholipid molecules of pro-liposome make them excellent *in vivo*.

Liposome as carrier of antigens

In addition their adjuvant effect, liposomes have been recognized as efficient carrier to deliver Biologically active material to specific cells. However, on administration of liposomes the major fraction is taken up by the liver and spleen unless steps are taken to retard their uptake. The following criteria help in successful homing of liposomised agent to target cells.

- Rate of uptake of liposome by RES must be minimized by using small, neutral, unilamellar liposomes having higher Tc and cholesterol.
- By coupling the surface of liposomes which would render liposomes less recognizable by RES.

- Coupling appropriate molecules (ligands) on the liposome surface which can bind to their receptors on the surface of target cell.

EVALUATION OF PROLIPOSOMES

Scanning electron microscopy (SEM)

Scanning electron microscopy is used to observe surface structure of the PL powder. which includes the comparison of the images of liposome and pure carrier material. The Carrier material in the formulation confirms the disposition of phospholipids on the carrier and thus proliposomes formulation confirms.^[34]

Transmission electron microscopy (TEM)

Transmission electron microscopy is used to check the structure of liposome after proliposomes powder hydration. In this process the powder of proliposome is hydrate with distilled water and then view lamellarity and the shapes under microscope.^[8,32,33]

Hydration study

Hydration study is carried out on the fact that liposomes are formed on contact with aqueous environment from proliposomes. In this method small quantity of dry powder of proliposomes are kept on a glass slide and then gradual addition of water is to be done. During hydration dissolution and disintegration occur rapidly. Liposome are formed when the water come in contact with the lipid surface of proliposomes. This process continuous tills the complete hydration lipid layer and carrier dissolution.^[35] formed vesicles are observed under microscopical method.

Zeta potential

Surface charge of proliposomes can be determined by the zeta potential. Zeta potential is the potential difference between electro neutral region of the solution and surface of tightly bound layer (shear place).^[36]

Flow property

Content uniformity and handling processing operation can be examined by the flow property of a powder formulation. For a solid powder formulation like proliposomes it is necessary to analyse the property. It can be assessed by measuring given parameters; Carr's Index, Angle of repose and Hausner's ratio.^[35,36]

CONCLUSION

Proliposomes possess excellent shelf life as compared to liposome and it offers non-invasive delivery of drug into or across the skin. Since better stability of liposomes *in vitro* can be achieved by proliposomal drug delivery systems. The proliposomes would be a better choice of preparation method. Innovation in drug delivery systems such as proliposomes has initiated a new area in vesicular research for topical drug delivery. Various reports & surveys show a promising future of proliposomes in making transdermal delivery of various agents.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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