TOPICAL LIPOSONAL GEL: A NEW STRATEGY OF NOVEL DRUG DELIVERY

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ABSTRACT

Liposomes established themselves as a promising novel drug delivery vehicle in several different basic sciences and as a viable alternative in several applications. Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shells consisting of lipids arranged in a bilayer configuration. Liposomes are acceptable and superior carriers having ability to encapsulate hydrophilic and lipophilic drugs and protect them from degradation. It also has affinity to keratin of horny layer of skin and can penetrate deeper into skin and hence give better absorption. Applied on the skin, liposomes may act as a solubilizing matrix for poorly soluble drugs, penetration enhancer as well as local depot at the same time diminishing the side effects of these drugs. Topical liposome formulations could be more effective and less toxic than conventional formulations. The liposome gel formulations could perform therapeutically better effects than the conventional formulations, as prolonged and controlled release topical dosage forms, which may lead to improved efficiency and better patient compliance.

KEYWORDS: Liposomal gel, novel drug delivery, topical application.
INTRODUCTION
Liposomal gel formulations are widely used to enhance the drug delivery efficiency through various routes of administration and have shown to be significantly superior to the other conventional dosage forms especially for the topical and intravenous administration.[1] However, therapeutic applications of systemically administered liposomes have been limited by their rapid clearance from the blood stream and their uptake by reticuloendothelial system (RES) in liver and spleen. The factors like pH, bile salt and pancreatic enzymes in the gastrointestinal tract may affect the liposome so that the use of liposomes for oral administration is not effective but the limitation can be minimized by the topical application for developing a novel drug delivery system.

Liposomal formulations are widely used in the pharmaceutical field as drug delivery systems due to their versatility and clinical efficacy and they have been used to administer drugs by several routes. Liposomes are having the higher diffusivity in the skin compared to most bare drugs so the liposomal formulations are widely used as topical drug delivery system.[2]

Liposomes are the spherical self-closed structures, composed of curved lipid bi-layers, which enclose part of the surrounding solvent into their interior. The size of a liposome ranges from 20 nm to several micrometers and they may be composed of one or several concentric membranes. A sustained release of encapsulated drugs makes the liposomes useful as the targeted drug delivery system. The adverse effect of the drugs can be minimized by controlling the permeability of the liposome membrane and thus avoiding the release of drugs.

Liposomes are incorporate into gel to enhance the skin retention of drugs and provide higher and sustained concentrations of drug in skin at the same time do not enhance the systemic absorption of drugs. They act as drug reservoir that provides a localized and controlled drug delivery. By the liposomal gel approach sufficient amount of drug can be delivering into skin so that the adverse effects of drug can be minimize. Carbapol is used as hydrogel which acts as a vehicle for liposomes have ability to enhance local delivery of drugs. The release of drug is controlled by degradation of hydrogel matrix. One can control release rate of the drug by tailoring the hydrogel degradation.
Classification of liposomes

Liposomes are classified as below.

A. Classification based on size of liposomes
1. Small Unilamellar vesicles.
2. Medium sized unilamellar vesicles.
3. Large unilamellar vesicles.
4. Giant unilamellar vesicles.
5. Unilamellar vesicles.
6. Oligolamellar vesicles.
7. Multilamellar vesicles.
8. Multivesicular vesicles.

B. Classification based on method of preparation
1. Vesicles prepared by extrusion method.
2. Vesicles prepared by French press.
3. Vesicles prepared by Fusion.
4. Vesicles prepared by reverse phase evaporation.
5. Frozen and thawed MLV.
6. Dehydration and rehydration vesicles.
7. Stable plurilamellar vesicles.

C. Classification based on composition and in-vivo application
1. Cationic liposomes
2. Temperature sensitive liposome
3. pH sensitive liposome.
4. Targeted liposomes
5. Long circulating liposomes.

**Advantages of liposome**
1. Liposome increases efficacy and therapeutic index of drugs.
2. Liposome increase stability of drug via encapsulation.
3. Increased solubility of the drugs.
4. The drug can be incorporated without any chemical reaction.
5. Targeted and controlled drug delivery is possible.

**Method of preparation of liposome**[^3-8]

The liposome preparation method depends on the various factors like the physicochemical characteristics of the material to be entrapped and those of the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and toxicity, optimum size of the vesicles formed, batch to batch reproducibility and possibility of large scale production of safe and efficient liposomal production.[3, 4]

The main structural components of liposome are phospholipids and cholesterol.

Phospholipids are the main structural component of the biological membrane such as the cell membrane. Phospholipids are can be classified by two categories like phosphoglycerides and sphingolipids. Phosphatidylcholine is mainly used for the preparation of liposomes due to its amphipathic molecule having hydrophilic polar head group phosphocholine, a glycerol bridge and pair of hydrophobic acyl chains.

Another structural component of liposome is the cholesterol. Incorporation of the sterol in the lipid bilayer brings about major changes in the preparation of the membrane. Cholesterol acts as a fluidity buffer, i.e. below the phase transition temperature it makes the membrane less ordered and slightly more permeable and above the phase transition temperature the membrane more ordered and stable.

**1. Film-hydration method**

It is the simplest procedure for the liposome formation but is limited because of its low encapsulation efficiency. This method produces liposomes by hydrating thin lipid films deposited from organic solution on a glass wall by shaking at temperatures above $T_c$.

[^3-8]: References [3-8]
(Transition temperature). The solvent is removed at reduced pressure in a rotary evaporator. The dry film of lipids which has been deposited onto the wall of a round bottom flask is hydrated by adding a buffer solution with a war soluble marker. As the lipid becomes hydrated and starts to form into closed vesicles only a small amount of the solute becomes entrapped. This method yields a heterogeneous sized population of MLVs over 1µm in diameter. Then the prepared liposome is further sonicated by using sonicator to form SUV.

![Figure 2: Preparation method of liposome by thin film hydration.](image)

2. **French pressure cell method**

French pressure cell method involves the extrusion of MLV at 20,000 psi at 4\(^\circ\)C through a small orifice. The method has several advantages over sonication method. The method is simple, rapid, and reproducible. The liposomes are formed by this method is quite larger than sonicated method. The limitation of this method is mainly difficult to achieve and the working volumes are relatively small.

3. **Solvent dispersion method**

   **A. Ether injection method**

A solution of lipids is firstly dissolved in diethyl ether or ether/ methanol mixture is slowly injected to an aqueous solution of the material to be encapsulated at pre determined temperature 55- 60 (\(^\circ\)C) or under reduced pressure. The subsequent removal of ether under vacuum leads to the formation of liposomes. The limitation of the method are population is heterogeneous and the exposure of compounds to be encapsulated to organic solvents or high temperature.\(^{[7]}\)
B. Ethanol injection method
A lipid solution of ethanol is injected to a buffer. The liposomes are formed rapidly. The main limitations of the method is same as ether injection method and also the liposomes are very dilute, it is difficult to remove all ethanol because it forms azotrope with water and the possibility of various biologically active macromolecules to inactivation in the presence of even low amounts of ethanol.

C. Reverse phase evaporation method
At first the emulsion is formed by the sonication method of a two phase containing phospholipids in organic solvent and aqueous buffer. Then the organic solvents are removed by applying negative pressure which results the formation of viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. The high encapsulation efficiency can be achieved by this technique. Several macromolecules can be encapsulated by this method.[8]

4. Detergent removal method
The detergents are used to solubilise the lipids. As the detergents are removed the micelles become progressively richer in phospholipid and finally combine to form LUVs. The detergents can be removed by dialysis method. The method is highly reproducible method and the liposomes prepared by this method are homogeneous in size. The limitation of the method is trace amounts of detergent can be present in the liposomal vesicles.

5. Freeze- thaw extrusion method
In this method liposomes formed by the film method are vortexed with the material to be encapsulated until the entire film is suspended and the resulting MLVs are frozen in a dry ice or acetone bath, thawed in lukewarm water and vortexed again. After completion of two additional cycles of freeze- thaw and vortexing the sample is extruded three times. This is followed by six freezes – thaw cycles and additional eight extrusions. The resulting vesicles are called large unilamellar vesicles by extrusion method. Proteins can be encapsulated by this method.

Incorporation of liposomes into the gel matrix[8]: Liposomes can be incorporated into gel to enhance their retention time on the skin. The liposomal gel provides higher and sustained concentrations of drug in skin at the same time do not enhance the systemic absorption of drugs. Hence adverse effects of many drugs can be minimized.
Evaluation of liposome\cite{9-14}

Liposomal formulation and processing for specified purpose are characterized to ensure their predictable in vitro and in vivo performance. The characterization parameters for purpose of evaluation could be classified into three broad categories.

1. **Structural characterization**: Physical characterization evaluates the vesicles size, shape and size distribution by light microscopy, fluorescent microscopy, electron microscopy, laser light scattering photon correlation spectroscopy, field flow fractionation, gel permeation and gel extrusion. By the electron microscopy like SEM (Scanning electron microscopy) and TEM (Transmission electron microscopy) the size of the liposomal vesicles can precisely characterized. Laser light scattering is one of the simplest methods to determine the size and the size distribution of the vesicles. The only drawback is the average property of bulk of the liposome cannot be determined.

Vesicle shape can be assced using electron microscopic techniques. Lamellarity of vesicles i.e. number of bilayers presents in liposome is determined using FE-SEM (Freeze fracture electron microscope) and P31 NMR (Nuclear magnetic resonance) analysis.

![Liposomal vesicle under SEM](image)

**Figure 3: Liposomal vesicle under SEM (Scanning electron microscope).**

2. **Entrapment efficiency**\cite{16}

Drug associated with liposome was separated from unentrapped drug using centrifugation method. Liposomes were centrifuged at 20000 rpm for 1 h at controlled temperature of 4°C. Supernatant containing unentrapped drug was withdrawn and measured UV spectrophotometrically against phosphate buffer saline (pH 7.4). The amount of drug entrapped in liposome was determined as follow

\[ EE(\%) = \frac{(C_d - C_f)}{C_d} \times 100 \]
Where Cd is concentration detected of total drug and Cf is concentration of free drug. The entrapment efficiency was obtained by repeating the experiment in triplicate and the values were expressed as mean standard deviation.

3. Optical microscopy
The microscopic method includes use of bright field, phase-contrast microscope and fluorescent microscope and is useful in evaluating vesicle size of large vesicles.

4. Laser light scattering photon correlation spectroscopy (PCS)
It is an evaluation of time dependence of intensity fluctuation in scattered laser light due to brownian motion of vesicles in suspension. A small particle diffuses faster than the larger one so the rate of fluctuation of scattered light intensity varies. By using stoke-einstein equation the translational diffusion coefficient (D) can be calculated.

5. Zeta potential
Zeta potential is the useful evaluation method to determine the charge of the liposomal vesicles. Zeta potential is the charge at the vesicle mobile surface and used to evaluate the degree of flocculation or de-flocculation in liposomes.

6. In-vitro skin permeation analysis
A biological membrane is used as a model membrane for the skin permeation study because of its similarity to human skin in lipid content and permeability. The skin samples were mounted between the two half-cells of a side-by-side diffusion chamber with a 37°C water jacket to control the temperature. The dorsal surface of the skin was placed in contact with the donor chamber, which was filled with the liposome formulation. The receptor chamber was filled with dissolution medium and stirred with a star-head Teflon magnetic bar driven by a synchronous motor. At time intervals of sample solution of receptor was withdrawn, and the same volume of fresh medium was added back into the chamber. The concentration of material which has been encapsulated in the samples was analyzed by suitable analytic technique and the cumulative amount of permeation was plotted against time.

7. Stability studies
The ability of vesicles to retain the drug (i.e., drug retentive behaviour) was assessed by keeping the liposomal suspensions and liposomal gel at two different temperature conditions, i.e., 4-8 °C (Refrigerator; RF), 25±2 °C (Room temperature; RT), for a period of 60 days.
Samples were withdrawn periodically and analysed for the drug content and particle size for liposomal suspension and drug deposition for liposomal gel in the manner described under entrapment efficiency and particle size distribution studies.

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2. Celadrin® is a registered trademark of Imagenetix, Inc.

b) OptisomeTM – Encapsulated Tetracaine.

c) Lipo CTM Liposome - encapsulated Active Vitamin C with Vitamin E and Zinc.

d) Lipo-Gest™ Natural Balancing Cream

e) Liposome progesterone based cream.

**CONCLUSION**

By the above study we can conclude that hydrophilic as well as lipophilic drugs can be easily encapsulate in liposomal formulation and dispensing in the form of carbopol gel was found to be well suited and sound approach to obtain stable liposomal formulation. Liposomal dispersion and gels were found to increase the skin permeation and deposition compared to other conventional forms. Liposomal gel technology offers entrapment of ingredients and thus reduced side effects, improved stability, increases elegance and enhanced formulation flexibility.

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