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Review Article

TOPICAL LIPOSOMAL GEL: A NOVEL DRUG DELIVERY SYSTEM

Nikhil Argan*, SL. Harikumar and Nirmala

Rayat and Bahra Institute of Pharmacy, Sahauran, District Mohali, Punjab, Chandigarh, India.

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 solublizing 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.

INTRODUCTION

Liposomes

Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shell(s) consisting of lipids arranged in a bilayer configuration. The potential use of liposomes as drug carriers was recognized more than 25 years ago and, since that time, liposomes have been used in a broad range of pharmaceutical applications.[1]

They present many advantages since they can be used as carriers for both hydrophilic and lipophilic molecules, as well as drug delivery systems for controlled drug delivery for different therapeutical purposes[2-5].An important aspect of liposomes is the protection that they afford as an encapsulating agent against potentially damaging conditions in external environments. Liposomes are also an important system in their own right in medical, cosmetic, and industrial applications[6-7].Liposomes can substantially improve drug loading, drug delivery and sustained release, thereby offering clear-cut advantages over traditional dosage forms[8].Liposomes were first produced in England in 1961 by Alec D.

Bangham, who was studying phospholipids and blood clotting[9]. It was found that phospholipids combined with water immediately formed a sphere because one end of each molecule was water soluble, while the opposite end is water insoluble. Water-soluble medications added to the water were trapped inside the aggregation of the hydrophobic ends; fat-soluble medications were incorporated into the phospholipid layer[10-11].

Topical drug delivery is an attractive route for local and systemic treatment[12].Liposomes are acceptable and superior carriers and have ability to encapsulate hydrophilic and lipophilic drugs[13-14]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. In the formulation of topical dosage forms, attempts are being made to utilize drug carriers that ensure adequate localization or penetration of drug within or through the skin in order to enhance the local and minimize the systemic effects or to ensure adequate percutaneous absorption[15]. Applied on the skin, liposomes may act as a solublizing 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. Skin has been considered as a promising route for the administration of drugs because of its accessibility and large surface area. Transdermal drug delivery system, designed to deliver a variety of drugs to the body through

diffusion across the skin layers, is appealing for several reasons including avoidance of the variable absorption and metabolic breakdown associated with oral treatments, drug administration can be continuous, and minimal intestinal irritation can be avoided. Liposome is has been used in transdermal drug delivery system because of its much higher diffusivity in skin compared to most bare drugs [16-18].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 such as the oral, parenteral, and topical. Among these, topical delivery of drugs carried by liposomes exhibits interesting applications, not only for promoting dermal delivery of drugs which have to act topically, such as local anaesthetics, but also for enhancing transdermal delivery of drugs intended for systemic use, thus more effectively exploiting this non-invasive alternative route to oral administration. Due to the forementioned advantages, in this study liquid-state liposomes were chosen to serve as the drug delivery system[19-20].Although liposomes demonstrated promise for Transdermal drug delivery, the practical application of these formulations onto the skin is less. However, these can be incorporated into the gels than can apply onto the skin. It has been found that liposomes incorporate into the gels are stable[21-22].Hydrogels are clinically acceptable systems that offer many advantages, such as suitable rheological properties, good tissue compatibility and convenience in handling and ease of application. Carbopol gels are approved for pharmaceutical use in several different administration routes. Cutaneous use of these gels is advantageous as they possess good rheological properties resulting in long residue times at the site of administration and they provide higher and sustained skin concentrations of drugs compared to conventional gels and creams. Moreover, carbopol gels are anionic hydrogels with good buffering capacity, which may contribute to the maintenance of the desired pH[23-24].

Classification of liposomes based on structure parameters[25]

1. Multilamellar Large vesicles > 0.5um
2. Oligolamellar vesicles 0.1-1um
3. Unilamellar vesicles (All size range)

a) Small unilamellar vesicles 20-100nm
b) Medium sized unilamellar vesicles
c) Large unilamellar vesicles >100nm
d) Giant unilamellar vesicles >1um

4. Multivesicular vesicles >1um

Advantages

1. Precipitation at the injection site and in the blood circulation can be prevented.
2. Phospholipids are one of the few solubilizers that are well tolerated intravenously.
3. Provide selective passive targeting to tumour tissues.
4. Increase safety and therapeutic index.
5. Increase stability via encapsulation
6. Site avoidance effect.

Methods of preparation of liposomes

A) Multilamellar Liposomes (MLV)

1. Lipid hydration method[26]

This is the most widely used method for the preparation of MLV. The methodinvolves drying a solution of lipids so that a thin film is formed at the bottom of roundbottom flask and then hydrating the film by adding aqueous buffer and vortexing thedispersion for some time. The hydration step is done at a temperature above the gel-liquidcrystalline transition temperature Tc of the lipid or above the Tc of the highest meltingcomponent in the lipid mixture. The compounds to be encapsulated are added either toaqueous buffer or to organic solvent containing lipids depending upon their solubilities.MLV are simple to prepare by this method and a variety of substances can beencapsulated in these liposomes. The drawbacks of the method are low internal volume,low encapsulation efficiency and the size distribution is heterogeneous.

2. Solvent Spherule Method[27]

A method for the preparation of MLVs of homogeneous size distribution .The process involved dispersing in aqueous solution the small spherules of volatile hydrophobic solvent in which lipids had been dissolved. MLVs were formed when controlled evaporation of organic solvent occurred in a water bath.

B) Small Unilamellar Liposomes (SUV)

1. Sanitation Method[28]

Here MLVs are sonicated either with a bath type sonicator or a probe sonicator under an inert atmosphere. The main drawbacks of this method are very low internal volume/encapsulation efficiency, possibly degradation of phospholipids and compounds to be encapsulated, exclusion of large molecules, metal contamination from probe tip and presence of MLV along with SUV.

2. French Pressure Cell Method[29]

The method involves the extrusion of MLV at 20,000 psi at 4°C through a smallorifice. The

method has several advantages over sonication method. The method issimple rapid, reproducible and involves gentle handling of unstable materials. The resulting liposomes are somewhat larger than sonicated SUVs. The drawbacks of the method are that the temperature is difficult to achieve and the working volumes are relatively small (about 50 mL maximum).

C) Large Unilamellar Liposomes (LUV)
They have high internal volume/encapsulation efficiency and are now days being used for the encapsulation of drugs and macromolecules.

1. Solvent Injection Methods
a) Ether Infusion Method[30]
A solution of 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 main drawbacks of the method are that the population is heterogeneous (70-190 nm) and the exposure of compounds to be encapsulated to organic solvents or high temperature.

b) Ethanol Injection Method[31]
A lipid solution of ethanol is rapidly injected to a vast excess of buffer. The MLVs are immediately formed. The drawbacks of the method are that the population is heterogeneous (30-110 nm), liposomes are very dilute, it is difficult to remove all 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.

c) Reserves Phase Evaporation Method[32-34]
First water in oil emulsion is formed by brief sonication of a two phase system containing phospholipids in organic solvent (diethylether or isopropylether or mixture of isopropyl ether and chloroform) and aqueous buffer. The organic solvents are removed under reduced pressure, resulting in the formation of a viscous gel. The liposomes are formed when residual solvent is removed by continued rotary evaporation under reduced pressure. With this method high encapsulation efficiency up to 65% can be obtained in a medium of low ionic strength for example 0.01 M NaCl. The method has been used toencapsulate small, large and macromolecules. The main disadvantage of the method is the exposure of the materials to be encapsulated to organic solvents and to brief periods of sonication. These conditions may possibly result in the denaturation of some proteins or breakage of DNA strands. We get a heterogeneous sized dispersion of vesicles by this method. Modified Reverse Phase Evaporation Method was presented and the main advantage of the method is that the liposomes had high encapsulation efficiency (about 80%). The Reverse Phase Evaporation has also been modified to entrap plasmids without damaging DNA strands.

d) Calcium-Induced Fusion Method[35]
This method is used to prepare LUV from acidic phospholipids. The procedure is based on the observation that calcium addition to SUV induces fusion and results in the formation of multilamellar structures in spiral configuration (Cochleate cylinders). The addition of EDTA to these preparations results in the formation of LUVs. The main advantage of this method is that macromolecules can he encapsulated under gentle conditions. The resulting liposomes are largely unilamellar, although of a heterogeneous size range. The chief disadvantage of this method is that LUVs can only be obtained from acidic phospholipids.

e) Freeze-Thaw Method[37]
SUVs are rapidly frozen and followed by slow thawing. The brief sonication disperses aggregated materials to LUV. The formation of unilamellar vesicles is due to the fusion of SUV during the processes of freezing and orthawing. This type of fusion is strongly inhibited by increasing the ionic strength of the medium and by increasing the phospholipid concentration. The encapsulation efficiencies from 20 to 30% were obtained.

(D) Giant Liposomes[38]
(i) The procedure for the formation of giant liposomes involves the dialysis, of a methanol solution of phosphatidylcholine in the presence of methylglucoside detergent against an aqueous solution containing up to 1 M NaCl. The liposomes range in diameter from 10 to 100 mm.

Preparation of Liposomal Gel[39]
Gel was prepared using carbopol® 934NF (1, 1.5 and 2%). The appropriate quantity of carbopol 934 powder was dispersed into distilled water under constant stirring with a glass rod, taking care to avoid the formation of indispersible lumps and allowed to hydrate for 24 h at room temperature for swelling. Topical liposome gel formulations were prepared by incorporation of liposome's containing drug

(separated from the unentrapped drug) were mixed into the carbopol gel with a mechanical stirrer (25 rpm, 2 m). The dispersion was neutralized using triethanolamine (0.5% w/w). Control gels were made under the same conditions.

Chracterization of Topical Liposomal Formulation

Size distribution[40]

Prepared liposomal batches were monitored for their morphological attributes using optical microscope. Mean vesicle size and size distribution profile of liposome was determined by using Malvern particle size analyzer model SM 2000, which follows Mie's theory of light scattering. Diluted liposome suspension was added to the sample dispersion unit containing stirrer and stirredat 2000 rpm in order to reduce the interparticle aggregation, and laser obscuration range was maintained between 10-20%. The average particle size was measured after performing the experiment in triplicate.

Entrapment efficiency[41]

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 (%) = $[(C^d–C^f)/C^d] 100$

Where C^d is concentration detected of total drug and C^f 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.

Zeta potential (z) determination[42]

Charge on empty and drug loaded vesicles surface was determined using Zetasizer 300HSA (Malvern Instruments, Malvern, UK). Analysis time was kept for 60 s and average zeta potential and charge on the liposome was determined.

Skin permeation and drug deposition studies

Rat was sacrificed by exposing to excess chloroform. Tothe abdominal skin, depilatory (Anne French, India) wasapplied and kept for 10 m to remove the hair from theskin. After 10 m of application, skin was washed withwater. Skin was excised from rat with scalpel and fattylayer was removed by keeping the skin in warm water at 60°c. After 2 m, fatty layer was peeled off gently and skinwas washed with water and kept for saturation inphosphate buffer saline pH 7.4 for about 30 m before itwas used for permeation studies. Fresh skin was usedevery time. Skin permeation studies with drug containing liposome formulations were carried out usingabdominal rat skin, employing modified Franz-diffusion cells. The results obtained were compared with that ofnon-liposomal formulations of drug. The skin wasprepared by mounting on the receptor chamber withcross-sectional area of 3.91 cm^2 exposed to the receptorcompartment. The receptor compartment was filled with(22 ml) phosphate buffer pH 7.4. It was jacketed to maintain the temperature 37 + 0.5°C and was kept stirring at 50 rpm. Prior to application of formulations, the skin was allowed to equilibrate at this condition for 1 h. Liposomal or non-liposomal formulation (amount equivalent to 5 mg of drug) was applied uniformly on the dorsal side of skin. Aliquots of 2 ml were withdrawn periodically and replaced with same amount of saline solution to maintain the receptor phase volume at a constant level. The samples were quantified spectrophotometrically. For determination of drug deposited in skin, cell was dismantled after a period of 8 h and skin was carefully removed from the cell. The formulation applied on skin surface was swabbed first with phosphate buffer pH 7.4 and then with methanol. The procedure was repeated twice to ensure no traces of formulation are left onto skin surface. The skin was then cutinto small pieces and drug present in skin was extracted in phosphate buffer pH 7.4

Rheological studies[43,44]

While considering the stable liposome dispersion or any other delivery system they usually need to beincorporated into convenient dosage for to obtainformulation with desired semisolid consistency for easein topical and transdermal application. It is important andcontrols the flow properties to ensure product quality andeffectiveness of the production. It helps in selection of dermatological formulation that will progress to clinical efficacy. In present study liposomal gels were prepared using carbopol 934 as gelling agent. Rheological analysis of liposome loaded carbopol gels were performed using a stress control rheometer (Viscotech Rheometer , Rheological Instruments AB, Lund, Sweden), equipped with stress rheologic basic software, version 5, using cone-plate

geometry with a diameter of the cone being 25 mm and a cone angle of 10, operating in the oscillation and static mode. Rheological analysis was performed at room temperature. The following parameters were carried out for rheology measurement.

Oscillation stress sweep[45,46]

Dynamic oscillation stress sweep was performed to determine the linear viscoelastic region (LVR). LVR is the region where the elastic modulus (G') was independent of applied stress because destruction in thestructure of gels occurs at high shear stress. Analysis of viscoelastic material was designed not to destroy the structure so that measurement can provide the information about intermolecular and interparticle forces in the material. This test gives idea about the critical stress beyond which the sample may show significant structural changes, and therefore the consequent choice of the stress value to be used in other in other oscillation tests. The samples were exposed to increasing stress (0.5 to 150 Pa) at a constant frequency of 0.1 Hz. The three main parameters determined in this test were the storage modulus G', loss modulus G" and loss tangent tan! The end point of the linear viscoelastic region was determined as a stress, when the G' value was dropped 10% from the linear level that indicated a significant change in the structure gel samples.

Oscillation frequency sweep[47]

The samples were exposed to stepwise increasing frequency (0.1 to 100 Hz) at a constant stress in the field of LVR and elastic moduli (G') as well as viscous modulus (G?) were recorded against frequency.

Drug content and content uniformity

The gel sample (100 mg) was withdrawn and drug content was determined using UV spectrophotometer. Similarly, the content uniformity was determined by analyzing drug Concentration in gel taken from 3 to 4 different pointsfrom the container. In case of liposomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analysed by using UV spectrophotometer.

Stability studies[48]

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.

Drug Criteria for Topical Liposomal Drug Delivery System Which groups of substances are considered to be especially interesting for liposomal encapsulation in the field of dermatology?[49,50]

1. There are drugs which are known to have severe side-effects by the conventional way of topical administration, e.g. topicalglucocorticosteroids.
2. There are substances which normally are effective by systemic application but not by topical application, e.g. interferon.
3. There are drugs which only show insufficient effects when applied topically. E.g. hamamelis distillate.

Marketed Formulation of Topical Liposome[51]

a) Celadrin®

1. Celadrin® Topical Liposome Lotion- 4 oz.
2. Celadrin® is a registered trademark of Imagenetix, Inc.

b) Optisome™ – Encapsulated Tetracaine.

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

d) Lipo-Gest™ Natural Balancing Cream

e) Liposome progesterone based cream.

CONCLUSION

Generally speaking Liposomes have some advantages which make them look interesting as drug carriers for topically applied drugs. First, they are variable concerning size and surface properties and Second, They can act as sustained release depots, releasing encapsulated drugs of half-lives ranging from 0.6 to 11 days. Moreover a new generation of liposomes, the so-called "collagen modified liposomes" can moderate the liposome-liposome and the liposome-cell interaction due to their collagen surface properties. This indeed might mean a greater possibility to control the drug release. The topically applied liposomal formulations, particularly those prepared from lipid mixtures of composition similar to the stratum corneum, would be an effective delivery system for the treatment of skin diseases. Since these liposomal formulations provide sustained, enhanced

levels in deeper strata of the skin,they have the capacity to meter a sufficient quantity of drug into deeper tissue to treat the skin symptomology. Such metering should also reduce the incidence of undesirable side effects arising from systemic administration, or enhanced systemic absorption of drug.

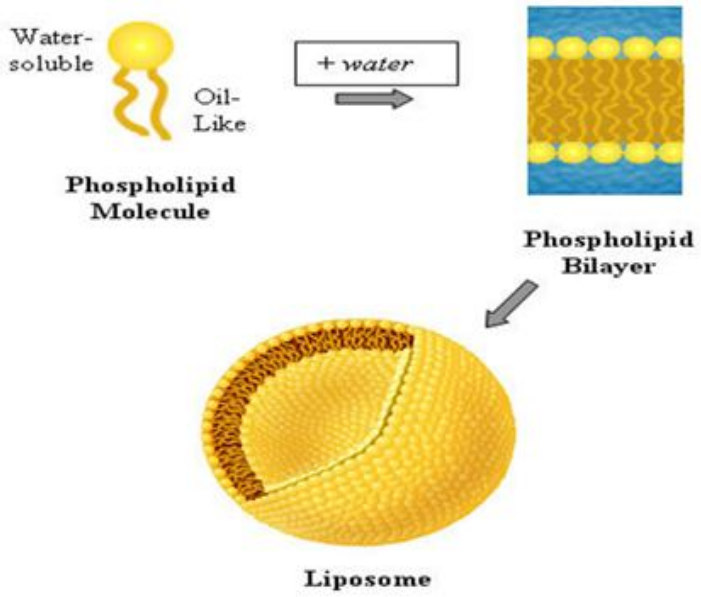

Fig. 1: Structure of Liposomes

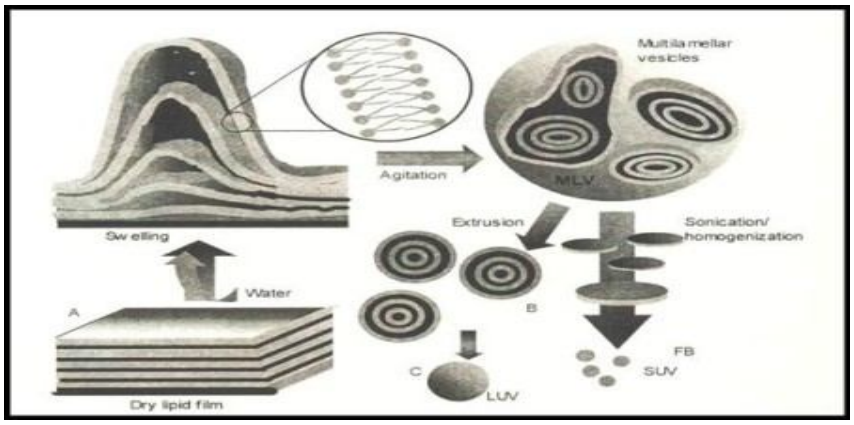

Fig. 2: Lipid Film Hydration Method

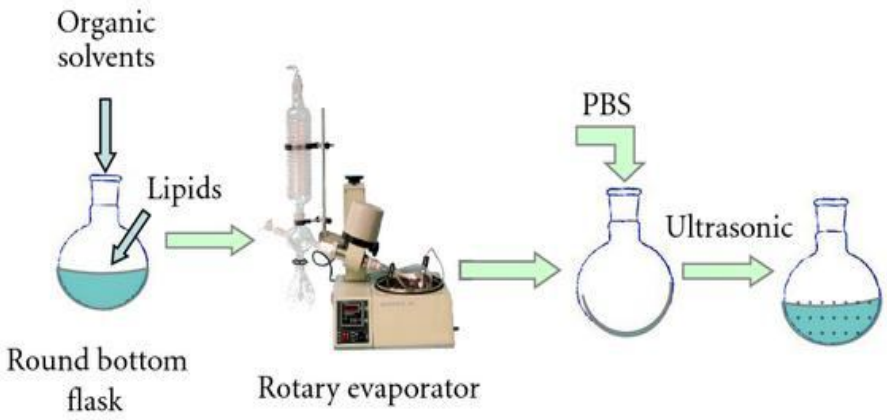

Fig. 3:

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