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PRONIOSOMES FOR IMPROVED TRANSDERMAL DRUG DELIVERY – A REVIEW

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ABSTRACT

Over the last few years comprehensive research has been done over provesicular approach for transdermal drug delivery. The transdermal route is very useful, but the stratum corneum acts as major barrier which is present on the top of the epidermis and behaves as a rate limiting membrane for penetration of drugs. The vesicular drug delivery system is potentially beneficial as the vesicles tend to fuse and adhere to the cell surface, thus increasing the permeability of the drug. Liposomes and niosomes are also vesicular system which can cross the stratum corneum but the major drawback is their instability. Proniosome is a dry formulation using suitable carrier coated with nonionic surfactant and can be converted into niosomes immediately before use by hydration. This proniosomes minimize the problems of niosomes and provide additional convenience in transportation, distribution, storage and dosing. Proniosomes can entrap hydrophilic as well as lipophillic drugs. Proniosomal gel offers a versatile vesicle drug delivery concept with potential for drug delivery via transdermal route. The focus of this article is to provide an overview on aspects related to mechanism of skin permeation of provesicular system; formulation, evaluation and application of proniosomal gel as a carrier for transdermal drug delivery.

Key Words: Proniosomes, Liposomes, Niosomes, Transdermal**.**

INTRODUCTION

Human skin is the important target site for the application of drug especially in the treatment of local disease. *[1, 2, 22]*

The transdermal route is widely used now days as it is convenient over the conventional dosage forms. Transdermal route bypasses the GI tract hence avoiding the gastric irritation, reduces number of doses, improved patient compliance, enhanced bioavailability and can maintain suitable plasma concentration. Vesicular drug delivery delivery is one of the approaches which encapsulates the drug eg. Liposomes, niosomes, transferosomes, pharmacosomes, and provesicles like proliposomes and proniosomes. *[3]*

Colloidal carriers have attracted the main interest because they are promising systems having a localized effect. These carriers accumulate in stratum corneum or other upper skin layers are not expected to penetrate into viable skin. *[4]* Despite of several researches, the barrier function of the stratum corneum still remains a major problem, which makes the development of new transdermal drug delivery systems an interesting challenge. *[5]* Penetration enhancement is the most critical factor in the transdermal drug delivery. In order for transdermal drug delivery systems to be effective, the drug must obviously be able to penetrate the skin barrier and reach its target in required concentration. Significant effort has been devoted to developing strategies to overcome the impermeability of intact human skin. These strategies include passive and active penetration enhancement and technologies to bypass the stratum corneum. Vesicular systems have been widely studied as vehicles for dermal and transdermal drug delivery. *[6,7]* A number of vesicle systems such as liposomes, niosomes, ethosomes, emulsomes and transfersomes have been developed.

Main problem regarding liposomes are as follows

- Degradation of liposome due to hydrolysis
- Sedimentation of liposome during storage.
- Aggregation of liposomes
- Fusion of liposomes
- Difficulties in sterilization

Payne et al, 1986 have introduced a new concept called ‘proliposomes’ which have better physical stability over liposomes due to their dry free-flowing nature. Proliposomes can be hydrated immediately before use. This dried form of liposomes consists of water soluble porous powder which acts as a carrier, loading of phospholipids and drug dissolved in organic solvent can be done. Another advantage is that, they can be sterilized and dispersed to form an isotonic multilamellar liposomal suspension.

Although proliposomal formulations are physically stable over liposomal formulations, a vacuum or nitrogen atmosphere is required for their preparation and storage to avoid oxidation of phospholipids. To avoid this technical difficulty, an alternative to phospholipids should be of great interest. *[8]*

One of the alternatives is to form liposomes like vesicles from nonionic surfactants like mono or dialkyl polyoxyethylene ether and cholesterol called 'niosomes' (10 to 1000 nm in size), which are quite stable and requires no special conditions like inert atmosphere for production and storage. *[9]* Niosomes are nonionic surfactant based multilamellar or unilamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulted from the organization of surfactants macromolecules as bilayers. *[10,11]* They are biodegradable, biocompatible and non immunogenic in nature. Niosomes have potential applications in the delivery of hydrophobic as well as hydrophilic drugs. *[12]* These vesicles are analogous to the liposomes (phospholipid vesicles) and serve as drug carrier because they can encapsulate both hydrophilic and lipophillic substrates. *[13]* Compared with the liposomes, niosomes offers higher osmotic stability with lower cost and greater availability of surfactants. *[14,15]* They improve the therapeutic performance of the drug molecules by delaying clearance from the circulation, protecting the drug from biological environment and restricting effects to target cells *[16]* Niosomes have industrial applicability due to their chemical stability and cost effective materials. Although, niosomes exhibit good chemical stability, they are physically less stable. An aqueous suspension of niosomes exhibit aggregation, fusion, leaking of the entrapped drug thus reduces the shelf life of dispersion. *[9]*

Hence, 'dry niosomes' can be prepared which are often called as 'Proniosomes' avoids many problems associated with niosomes like physical stability. proniosomes can be hydrated immediately before use to give niosomal dispersion. Proniosomes are dry, free flowing granular product which upon hydration gives a multilamellar niosomal dispersion. In addition convenience in transport, storage, and dosing makes proniosomes as a promising carrier. Proniosomes are provesicular approach which overcomes the limitations of other vesicular drug delivery (liposomes and niosomes). This proniosomal drug delivery has attracted towards transdermal delivery because surfactants themselves act as penetration enhancers and are biodegradable, non-toxic, amphiphillic, possess property of encapsulation and they can entrap both hydrophilic as well as lipophillic drugs. Proniosomes can be converted into niosomes in-situ by absorbing water from

the skin. Hence proniosomes serves as a promising carrier for transdermal delivery. *[17]*

Studies mostly focused on utilization of proniosomes in transdermal drug delivery.

Advantages of proniosomes:

1. Proniosomes have potential for the entrapping wide range of active compounds.
2. Convenient for transportation, sterilization, distribution, storage and dosing.
3. Problem of degradation by hydrolysis or oxidation which is usually seen in liposomes is avoided.
4. Requires no special conditions for storage and handling.
5. Sedimentation, aggregation or fusion is not seen.
6. Uses acceptable solvents in the preparation. *[18]*

STRUCTURE OF PRONIOSOMES

Proniosomes are present in transparent, translucent or a semisolid gel structure. Because of limited solvent presence, formed proniosomes is a mixture of phases of liquid crystal like lamellar, hexagonal, cubic as shown in figure-1. Here, the lamellar phase showed sheets of surfactant arranged in bilayer, hexagonal phase showed the cylindrical compact structure arranged in a hexagonal fashion whereas cubic phase consist of curved continuous lipid bilayer extending to three dimensions. While formulating this gel, in the beginning, less viscous composition is formed in some cases but the addition of water leads to interaction between water and a polar group of surfactant resulting swelling of bilayers. If the amount of solvent is increased further, then a spherical structure is formed i.e. multilamellar, multivescicular. Complete hydration leads to the formation of 'niosomes'. *[19, 20]*

Figure-1: Structure of Proniosome

MECHANISM OF DRUG PERMEATION OF VESICLES THROUGH SKIN:

Following types of vesicle-skin interactions are observed during in vitro studies using human skin.

1. Absorption and fusion of vesicles onto skin surface leading to increase in thermodynamic activity gradient of the drug at the interface, which act as driving force for absorption of lipophillic drugs across the stratum corneum.
2. Modification in the structure of the stratum corneum is also a type of interaction involves the ultra structural changes in the intracellular lipid region of the skin and its deeper layers which is revealed by Freeze Fracture Electron Microscopy (FFEM) and Small Angle X-ray Scattering (SAXS).
3. Bilayer present in niosomes act as rate limiting barrier for drugs.
4. Proniosomes contain both non-ionic surfactant and phospholipids, both can act as penetration enhancer and useful in increasing penetrability of many drugs.
5. The penetration enhancer effect of vesicles to reduce stratum corneum barrier properties.

Factors affecting the penetration of vesicles

1. Nature of drug
2. Size and composition of vesicles
3. Biophysical factors *[21,22]*

INTERACTION BETWEEN SKIN AND PRONIOSOMES:

There is a direct contact of proniosome formulated with skin after applying, so it is better to discuss the potential interactions between skin and vesicles formed in proniosome/niosome formulations. As we know that proniosomes or proniosomes derived niosomes are composed of non-ionic surfactants, and the vesicles are composed of these non-ionic surfactant only. So it is advisable to study the interactions between non-ionic surfactants and the skin. The nonionic surfactants are amphipathic molecules consisting of a hydrophobic (alkylated phenol derivatives, fatty acids, long chain linear alcohols, etc.) and a hydrophilic part (usually ethylene oxide chains of variable length). Nonionic surfactants are used widely in pharmaceuticals to increase their stability, solubility and permeation. There is a strong indication that the degree of interaction between vesicles and skin mainly depends on physicochemical properties of the surfactant molecules of which the niosomes or proniosomes are composed. Skin consists of a range of bioactive material like membrane

phospholipids, proteins, amino acids, peptides, etc. Surfactants are known to increase the permeability of vesicles and phospholipid membranes, causing low molecular mass compounds to leak. The interaction between biological membranes and non-Ionic surfactant tested for phospholipid composition and rate of biosynthesis of major phospholipid components indicate no significant change in the phospholipid composition, whereas biosynthesis and turnover rates of phospholipids were increased two to four times. *[23]*

MECHANISM OF VESICLE FORMATION IN PRONIOSOMES

Nonionic surfactants posses the ability to form bilayer vesicles which depend not only on Hydrophillic-Lipophillic Balance (HLB) of surfactant but also on a critical packing parameter (CPP). CPP can be defined as the relationship between structure of surfactant including size of hydrophilic head group and length of hydrophobic alkyl chain in the ability to form vesicles is described as

$$CPP = \upsilon / lca$$

Where, υ = the hydrophilic group volume,

lc=critical hydrophobic group length and

a= area of the hydrophilic head group.

As entrapment efficiency and particle size are inversely proportional to each other therefore CPP holds an important place in the formulation development. When the value of CPP is between 0.5 to 1, then surfactant is likely to form vesicles. CPP below 0.5 (indicates that there is a high contribution from the hydrophilic head group) gives spherical micelles and value of CPP above 1 (indicates that there is a high contribution from the hydrophobic group) gives inverted micelles which in later stages gives precipitation. Spans are most widely used in proniosomal preparation. All the grades of spans have same head group but are differentiated on the basis of alkyl chain. As per literatures, entrapment efficiency increases as alkyl chain length increase.

Span 60 (C18) > Span 40 (C16) > Span 20 (C12) >Span 80 (C18).

Span 60 and span 80 have same head group but there is a difference in the alkyl chain of span 80, which is unsaturated. Introduction of the double bond to the paraffin chain of span 80 causes marked enhancement of permeability, this may be the reason of low entrapment efficiency. On addition of cholesterol, tendency of surfactants to form aggregates is decreased. Cholesterol also provides stability to bilayer membrane by increasing gel liquid transition temperature of vesicle and also attributes to high HLB value and small CPP. Addition of lecithin, diacetyl hydrogen phosphate and

stearyl amine also enhances the stability and permeability of the bilayer. *[18]*

METHODS OF PREPARATION FOR PRONIOSOMES

Proniosomal formulations can be prepared by mainly three methods such as slurry method, slow spray coating method and coacervation phase separation method.

1. **Slurry method**

Proniosomes can be prepared from a stock solution of surfactants and cholesterol in a suitable solvent. The required volume of surfactant and cholesterol stock solution per gram of the carrier and drug should be dissolved in the solvent in a round bottom flask containing the carrier (maltodextrin or lecithin). Additional chloroform can be added to form the slurry in case of lower surfactant loading. The flask has to be attached to a rotary flash evaporator to evaporate solvent at 50- 60 rpm at a temperature of 45±20 C and a reduced pressure of 600mm of Hg until the mass in the flask had become a dry, free flowing product. Finally, the formulation should be stored in a tightly closed container under refrigeration in light. *[24-26]*

2. **Slow spray coating method**

A 100 ml round bottom flask containing desired amount of carrier can be attached to the rotary flash evaporator. A mixture of surfactants and cholesterol should be prepared and introduced into round bottom flash on rotary evaporator by sequential spraying of aliquots onto carrier's surface. The evaporator has to be evacuated and rotating flask can be rotated in water bath under vacuum at 65-70oC for 15 – 20 min. This process has to be repeated until all of the surfactant solution had been applied. The evaporation should be continued until the powder becomes completely dry. *[26-28]*

3. **Coacervation phase separation method**

This is widely used method for preparation of proniosomal gel. Proniosomal gel is basically mixtures of many phases of liquid crystals like lamellae, cubical or hexagonal which upon hydration formsunilameller to multilameller and spherical structures. Precisely weighed amount of drug, surfactant, lecithin, cholesterol take place and suitable alcohol is taken in clean, dry wide mouth glass vial and to it, 0.5 ml alcohol is added (minimum amount of alcohol is added so that micelle formation does not take place). All the ingredients are mixed well with the help of glass rod and covered with a lid to prevent loss of solvent. Further it is warmed on a water bath at 60-70°C for 5 min until all the surfactant dissolved completely. Then aqueous phase (glycerol, isotonic phosphate buffer or distilled water) is added in small amount so as to ensure only the gel formation and not the dispersion.

Again it is heated further for 2 min to give clear dispersion which on cooling to room temperature gives formation of proniosomal gel. In this formulation, water addition leads to swelling of bilayer due to interaction of water and polar groups of surfactant. *[18,26]*

Advantages:

1. Simple and easy method.
2. Specialized instrument is not required.
3. Small dose formulations can be prepared in lab scale.
4. Less time consuming.

CONVERSION OF PRONIOSOMES INTO NIOSOMES

Proniosomal gel is an intermediate state of formation of noisome. Less quantity of continuous phase(aqueous phase) gives formation of liquid crystalline compact mass of proniosomes. These formed proniosomes can be converted to niosomes by two ways

a. Hydration by skin- water in the skin is used as a hydrating medium for proniosomal gel which converts proniosomes to niosomes.
b. Hydration by a solvent - aqueous system like water, buffers, saline are used for conversion of proniosomes to niosomes with or without agitation.

The proniosomal gel is used for dermal and transdermal application. This formulation takes water from skin and converts to niosomes. After addition of the aqueous phase, agitation and sonication gives small size vesicular niosomes. This addition of aqueous media gives swelling of bilayers due to interaction of water with polar groups of surfactant. Due to inclusion of water into the bilayer, stacked structure tends to seperates. Above the limiting concentration of solvent, bilayers tend to form a spherical structure giving unilamellar or multilamellar vesicles (Fig 2). *[29]*

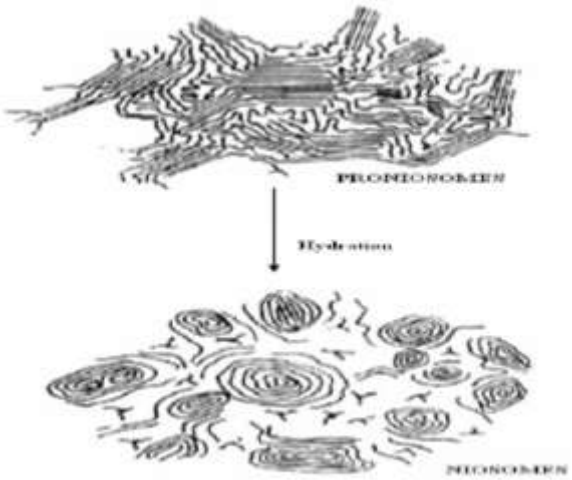

Figure-2: Proniosome to noisome conversion

FACTORS AFFECTING PHYSICAL NATURE OF PRONIOSOMES

There are some factors such as hydration temperature, choice of surfactant, nature of membrane, the nature of drugs, etc., can affect

significantly the physical nature of proniosomes (fig. 3). *[30]*

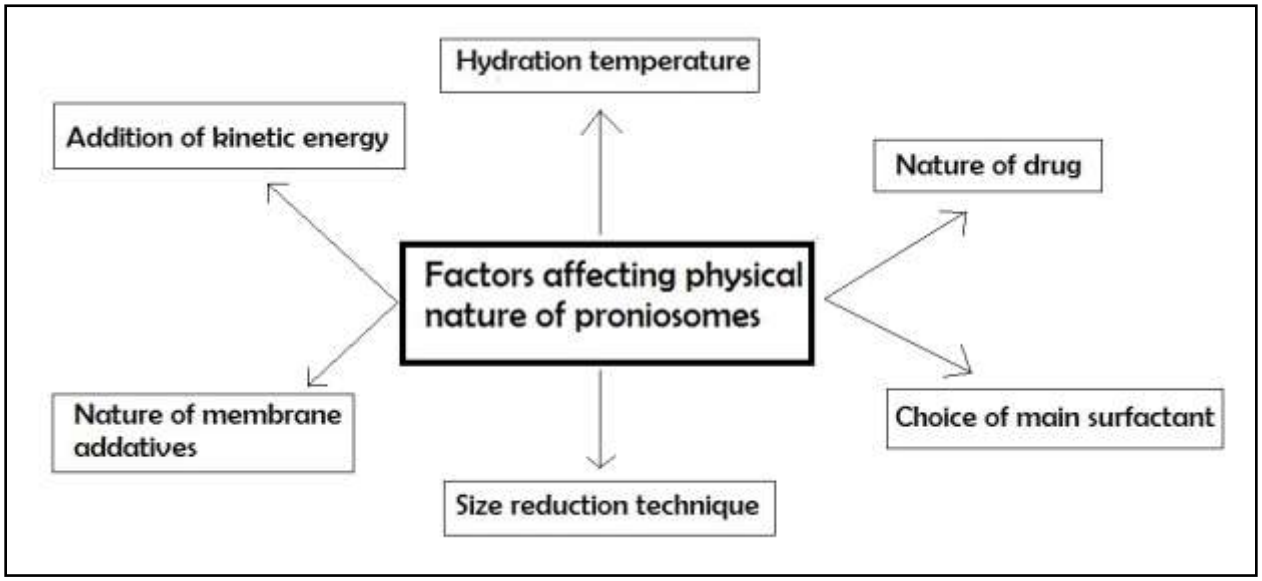

Figure-3: Factors affecting Physical nature of Proniosomes

FORMULATION ASPECTS OF PRONIOSOME

Proniosomal gel is comprised of ingredients like membrane stabilizer (lecithin, cholesterol), non-ionic surfactants (sorbitans and polysorbitans), alcohol and aqueous phase.

1. **Surfactants:**

Hydrophillc Lipophillic Balance (HLB) is the basis for the selection of surfactant. The HLB value indicates that the surfactant will form vesicle or not. It is reported thatthe HLB value between 4 and 8 are good candidates for vesicle formation. Hydrophillc surfactants, due to their high aqueous solubility on hydration, cannot attain a concentrated system in order to allow free hydrated units to exist aggregates and coalesced to form a lamellar structure . High HLB value reduces the surface free energy and allows vesicle formation of large size. Span 40 and span 60 have high HLB value, which results in reduced surface free energy, hence large size vesicles are formed, which gives a larger area exposed to skin and dissolution medium. HLB value and Phase Transition Temperature affects the encapsulation efficiency of surfactant. All spans have high Phase Transition Temperature hence good encapsulation efficiency, less leakage of drug. Encapsulation efficiency of tween is low as compared to spans. [21] List of surfactants is given in Table 1.

Table-1: Surfactants used in proniosome formulation

Sr no.	Name of surfactant	Use
1	Span 20	To increase drug flux rate across the skin .
2	Span 40	
3	Span 60	
4	Span 80	
5	Span 85	
6	Tween 20	
7	Tween 60	
8	Tween 80	

Chemical structure of surfactants influences drug entrapment efficiency. Increasing the alkyl chain length is leading to higher entrapment efficiency. It had also been reported that spans having highest phase transition temperature provides highest entrapment for the drug and vice- versa. Drug can be entrapped into proniosomes composed of tweens; however the encapsulation efficiency was relatively low as compared to those composed of spans. Most of surfactants used to make nonionic surfactant vesicles have a low aqueous solubility. However, freely soluble nonionic surfactants such as tween can form the micelles on hydration in presence of cholesterol. *[26]*

2. Lecithin:

lecithin as a complex mixture of acetone-insoluble phosphatides that consists chiefly of phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol, combined with various amounts of other substances such as triglycerides, fatty acids, and carbohydrates as separated from a crude vegetable oil source. The composition of lecithin (and hence also its physical properties) varies enormously depending upon the source of the lecithin and the degree of purification. Egg lecithin, for example, contains 69% phosphatidylcholine and 24% phosphatidylethanolamine, while soybean lecithin contains 21% phosphatidylcholine, 22% phosphatidylethanolamine, and 19% phosphatidylinositol, along with other components.

In proniosomal gel, lecithin plays important role like

- Lecithin acts as a penetration enhancer
- Increases entrapment efficiency due to high phase transition temperature
- Prevents leakage of the drug from vesicle
- Reduces vesicle size due to increase in the hydrophobicity (vesicle composed of soya lecithin is of larger size than that composed of egg lecithin)

Egg lecithin contains saturated fatty acid while soya lecithin contains unsaturated fatty acids,

oleic acid and linoleic acid, hence soya lecithin is having good penetrability over egg lecithin. *[3]*

3. Cholesterol:

Cholesterol is an important component of proniosomal vesicle. As it influences stability and permeability of vesicle. It was found that entrapment efficiency increase with increase in cholesterol content up to a certain limit, at higher concentration it has lowring effect on entrapment efficiency. This is because the cholesterol molecule act as vesicular cement which accommodates itself in the molecular cavities formed when surfactant monomers are assembled into bilayers to form niosomal membranes, this results in increased rigidity and decreased permeability as compared to cholesterol free niosomal membrane. On further increase in cholesterol concentration, it competes with drug for accommodation between bilayers and disrupts the regular structure of vesicular membrane. *[3]*

4. **Solvent:**

Alcohol used has great influence on vesicle size and permeability of the drug. Vesicles formed from different alcohols have different size and they follow the order

Ethanol > Propanol > Butanol > Isopropanol

Ethanol gives the highest size due to high aqueous solubility and lowest size with usual is due to branched chain present in it. Selection of solvent also affects the rate of spontaneity of formation of niosomes. Formulation in which isopropanol and butanol is used, niosomes are formed more spontaneously because of faster phase separation of isopropyl alcohol and butanol due to their low aqueous solubility. *[21]*

5. Aqueous phase:

0.1% Glycerol, phosphate buffer pH 7.4 or distilled water is used as an aqueous medium for preparation of proniosomal gel. Selection and pH of aqueous system affects the entrapment efficiency and particle size of proniosomes. *[21]*

6. Miscellaneous:

a. **Dicetyl Phosphate (DCP):** It is a charged molecules used to impart negative charge to the niosomal vesicles. Formulations containing DCP shows slightly greater amount of drug than those containing surfactant and cholesterol only. It is reported that drug release was maximum for the proniosomes containing DCP due to the charge present in the DCP containing bilayers, which is responsible for an increase in the curvature and decrease vesicle size. DCP decreases the entrapment efficiency of drug into vesicles.

b. **Stearylamine (SA):** This is a charged lipid used to impart a positive charge to the vesicle. SA decreases the entrapment efficiency.

c. **Solutan:** Solutan C24 a poly-24 oxyethylene cholesteryl ether, is added to formulations to give homogeneous nature and devoid of aggregates. *[21]*

FACTORS AFFECTING FORMULATION OF PRONIOSOMAL GEL

1. Surfactant chain length:

All span types have same head group but different alkyl chain. Increasing the alkyl chain length leads to higher entrapment efficiency. It follows the order Span60 (C18) >Span40 (C16) >Span20 (C12) >Span80 (C18). Span 60 and Span 80 have the same head groups but Span 80 has an unsaturated alkyl chain.

2. Cholesterol content:

Depending upon type of surfactant used, cholesterol can increase or decrease the encapsulation efficiency. Generally cholesterol gives intact bilayer formation which leads to reduced permeability of niosomal vesicle.

3. pH of hydrating medium:

Percent encapsulation efficiency of niosomes prepared by hydration of proniosomal gel with span 60 and cholesterol are greatly affected by pH of hydrating medium. For example, the fraction of flurbiprofen encapsulated was increased to about 1.5 times as the pH decreased from pH 8 to 5.5. The increase in the percentage encapsulation efficiency of flurbiprofen by decreasing the pH could be attributed to the presence of the ionizable carboxylic group in its chemical structure. Decreasing the pH could increase the proportions of the unionized species of flurbiprofen, which have higher partitioning to the bilayer lipid phase compared to the ionized species.

4. Total lipid concentration:

Percent encapsulation efficiency of flurbiprofen was increased with an increase in lipid concentration linearly. But, the amount of flurbiprofen entrapped was decreased.

5. Drug concentration :

Increasing flurbiprofen concentration from 25 to 75mg/mmol lipids in the proniosomes prepared from Span 60/cholesterol (9:1) showed an increase in both percentage encapsulation efficiency and the amount of drug encapsulated per mol total lipids upon hydration and formation of niosomes.

6. Charge of the lipid:

Incorporation of DicetylPhosphate (DCP) or StearylAmine (SA) which induces a positive and negative charge respectively, decreases percent

encapsulation efficiency of flurbiprofen niosomes. *[9]*

EVALUATION PARAMETERS OF PRONIOSOMAL GEL

1. **Vesicle size and shape:**

Proniosomes give niosomes upon hydration which are spherical in shape, their size, morphology is studied by the light microscope, electron microscope, SEM and TEM, photon correlation microscopy, freeze fracture electron microscopy (FFEM) *[31]*

2. **Entrapment efficiency (measurement of partitioning):**

The entrapped drug is separated by dialysis, centrifugation, freeze thawing, filtration or gel chromatography. Either entrapped drug is determined by complete destruction of vesicles (using 50% propane or 0.1% Triton) or unentrapped drug is measured and subtracted from total amount of the drug. Dialysis method is suitable for large vesicles (>10 µm) only. It is an extremely slow process and dilutes the niosomal dispersion. Centrifugation is fast method and also inexpensive but sometimes leads to destruction of the fragile system. Ultracentrifuge is a modern technique which sediments all size population. In gel filtration, Sephadex gel is used, it is also quick method but not suitable for highly viscous formulations. *[31]*

3. **Rate of spontaneity:**

Rate of spontaneity is defined as number of niosomes formed after hydration of proniosomes for 15 min. Here, around 0.2g of proniosomal gel is transferred to bottom of the small Stoppered glass tube and then spread along the walls of the container uniformly. Further, two ml saline(0.154 M NaCl) is added carefully and keep aside for 15 min without agitation. A drop of this aqueous layer is withdrawn and placed over Neubaur's chamber. Number of niosomes eluted from proniosomes is counted. *[31]*

4. **In-vitro drug release:**

In vitro drug release and skin permeation is determined by using different techniques like Franz diffusion cell, Keshary-Chein diffusion cell, Cellophane dialyzing membrane, Dialysis tubing or USP dissolution apparatus type 1. In all the above mentioned processes dialysis of the proniosomal gel is done against the buffer or other specified media at a specific temperature. Following method are given in the literatures to determine drug release from vesicles-

a. Diffusion cells are used to study the release rate of drug, generally a Franz diffusion cell is used. Here, dialysis membrane is mounted between donor

and receptor compartment. Specific amount of gel is placed over the membrane. Phosphate buffer saline pH 7.4 is taken in receptor compartment. This receptor compartment is surrounded by a water jacket to maintain the temperature at 37°C. Heat is supplied using a thermostat with magnetic stirrer. The receptor fluid is circulated by a Teflon coated magnetic bead. The specific amount of sample is withdrawn at the sampling interval and same volume is replaced with fresh receptor fluid. Cumulative percent release at the end of analysis is calculated.

b. Proniosomal gel is converted to niosomal dispersion by sonication and then this is poured in a dialysis bag. The bag is closed from both the sides and the assembly is placed at the bottom of the USP dissolution apparatus. Vessel contains 1000ml of buffer and speed is adjusted to 50 rpm. Aliquots are withdrawn at the sampling intervals from release medium and replaced by fresh medium. Amount of drug release is calculated at the end of the analysis.

c. Proniosomal gel is spread on the circular glass disc which is further covered with the cellophane dialysis membrane and securely mounted with the help of rubber bands. The disc is then placed on the bottom of the glass tube to accommodate the disc diameter and around 50 ml of dialysate is poured on membrane surface. This assembly is immersed into the water bath which is maintained at 37.8°C. Dialysate is continuously stirred using a motor or peristaltic pump. *[21].*

5. In vitro skin permeation:

For in vitro skin permeation, albino rat skin, Wister rat skin is used. Two types of cells are used for permeation study

a. Franz diffusion cell: The rat skin is mounted on the receptor compartment with stratum corneum facing to donor compartment. The donor compartment is filled with the proniosomal formulation. Top of the diffusion cell is covered with paraffin paper. Receptor compartment is maintained at 37°C using a thermostat with magnetic stirrer. At each sampling interval, samples are withdrawn from the receptor compartment and same volume is replaced with fresh medium. Aliquots are analyzed by UV spectrophotometer or HPLC.

b. Keshary Chein cell: Here proniosomal gel is applied to the furry side of the

skin. This prepared skin is mounted between donor and receptor compartment with furry side facing towards the donor compartment. The receptor fluid is maintained at 37°C using thermostat with magnetic stirrer. At each sampling interval, specific amount of receptor fluid is withdrawn and again replaced with fresh media. Cumulative percent release is determined. *[21]*

6. Stability study:

Stability study is carried out by storing prepared formulations at various temperature conditions like refrigeration temperature (2°-8°C), room temperature (25±0.5°C) and at elevated temperature (45°±0.5°C) for the period of one month. Formulation is evaluated for vesicle size, drug content and release rate periodically and also at the end of the analysis. *[21]*

APPLICATIONS OF PRONIOSOMES IN TDDS

The Proniosomal gel system is used for not only targeting the drug delivery but also used for sustained, controlled release and transdermal drug delivery. Proniosomal drug delivery also has an application in cosmetics. Proniosomes have improved bioavailability, reduced side effects and as vesicular membrane is similar to that of biological membrane which helps in enhancing the permeation of bioactive materials. *[32]*

1. **Applications in cardiovascular diseases**

Proniosomes are used as carrier for transdermal delivery of captopril for the treatment of hypertension. The study shows that this proniosomal system is capable of delivering the drug for an extended period of time. Encapsulation of the drug was done by using various sorbitan esters, cholesterol and lecithin. Transdermal proniosomal drug delivery is also done on losartan potassium, where sorbitan esters and sorbitan mono esters (spans and tweens), cholesterol and lecithin is used. Lisinopril dehydrate, is a orally active ACE inhibitor, considered for anti-hypertensive therapy which have only 50-60% bioavailability. Upon oxidation, lisinopril gives lisinopril disulfide, which is having a poor intestinal absorption. Lisinopril when administered initially cause hypotension, which can prove to be harmful in diuretic treated and congestive heart failure patients. Therefore, the use of the transdermal Proniosomal gel could reduce the side effects associated with oral route. Proniosomal gel is prepared with cholesterol; lecithin, surfactants and lisinopril dehydrate and further tested for evaluation parameters. Valsartan, ACE inhibitor, is rapidly absorbed following oral oral administration but have

bioavailability of only 23%. Proniosomal gel of valsartan is prepared and evaluated for vesicle size analysis, entrapment efficiency, diffusion studies and stability of the gel. *[32]*

2. Hormonal Therapy

Extensive work has been done on proniosome based transdermal delivery of levonorgestrel. The niosomal structure was liquid crystalline compact niosome hybrid. The system was tested for particle size, encapsulation efficiency, rate of spontaneity, polydispersity, stability study and in vivo, in vitro testing is performed. Biological assay for progestational activity included endometrial assay and inhibition of formation of corpora lutea. Various proniosomal formulations were tested for the skin permeation of estradiol. Particle size, entrapment efficiency, in vitro permeation is studied. *[32]*

3. Application in diabetics

Skin permeation mechanism with proniosomal gel of frusemide is performed, in which span, Soya lecithin, diacetyl phosphate and cholesterol are used. Overall findings suggested that the proniosomes serves as a non-invasive delivery of frusemide. *[32]*

4. NSAID Application and pain management

Ketorolac tromethamine (KT) is a non-steroidal anti inflammatory drug with potent anti-inflammatory and analgesic activity is administered orally or intramuscularly daily in divided multiple doses (due to short half life of about 4-6h) in the management of postoperative pain. This frequent dosing reduces patient compliance. Therefore transdermal delivery through proniosomes serves as a better alternative route for administration of KT to maintain the drug blood levels for an extended period of time. Piroxicam is also NSAID used in the treatment of rheumatoid and osteoarthritis is a potent analgesic. But, its oral administration leads to gastric irritation. Transdermal delivery needs deeper penetration of drugs. Proniosomes are used as carrier for delivery of poorly water soluble drug like Celecoxib. Here, proniosomal gel is prepared using span 40 and span 60, cholesterol and lecithin. This gel is also compared with the standard transdermal gel formulated in a carbopol 934 (1%w/v) base. Meloxicam, a nonselective NSAID, is recommended for rheumatoid and osteoarthritis, severely affects the GI tract when administered orally. Hence a proniosomal transdermal delivery reduces the problems and high local concentration is maintained at the local site. Tenoxicam ia also NSAID which is widely used in the treatment of rheumatic disorders, gout, enkylosing spondylitis and

dysmenorrheal. Its oral administration affects GI tract severly In addition, liver and billiary tract is also affected. Proniosomal gel serves as promising carrier for transdermal drug delivery of tenoxicam. Gugulipid is an ethyl acetate extract of guggul resin, obtained from Commiphora wightii (Fam.:Burseraceae) has wide range of therapeutic activities. But, has low bioavailability and low aqueous solubility, hence a proniosomal approach removes these undesired pharmacological actions and improves therapeutic concentration at the site of action. Guggulipid loaded proniosomal gel has been developed and characterized for particle size, entrapment efficiency, in vitro drug release and in vivo anti inflammatory activity. Proniosomes of flurbiprofen have been formulated and effect of formulation parameters on flurbiprofen release and encapsulation studied. Effect of cholesterol, total lipid concentration, pH of hydrating medium, influence of charge lipids is checked. *[32]*

5. Applications in psychosis

Proniosomal formulations with non-ionic surfactant for Haloperidol are studied. The effect of hydrophilicity and hydrophobicity of surfactants on drug solubility, proniosome surface structure and stability and skin permeation of haloperidol from different formulations have been investigated. Haloperidol (HP) was entrapped in proniosomes with very high efficiency for all formulations. Stability studies performed at 4 degrees C and 25 degrees C for a period of 6 weeks did not reveal any significant drug leakage. Interfacial tension and surfactant hydrophobicity appeared to be useful for elucidating mechanism of skin permeation and for comparing drug fluxes from different proniosomal formulations. *[32]*

6. Applications in cerebral degenerative disease

Vinpocetine is a poorly water-soluble vincamine derivative, is widely used for the treatment of disorders arising from cerebrovascular and cerebral degenerative diseases. Its clinical use through oral administration is limited by poor absorption, extensive first pass metabolism and extremely low bioavailability of only 7% hence it implies frequent dosing, which is inconvenient for patients with dementia. Hence to overcome these problems, proniosomal controlled transdermal strategy is used in which sugar esters are incorporated as permeation enhancers. *[32]*

CONCLUSION

Proniosomes have advantages of controlled and sustained release action, stability and versatility as a drug carrier. Proniosomes can be used as efficient carrier for various categories of drugs

with improved physical and chemical stability, good bioavailability for poorly soluble drugs. Proniosomes contain both non ionic surfactant and phospholipids, both can act as penetration enhancer and useful in increasing permeation of many drugs. Proniosomes are good candidates for transdermal delivery of drugs due to non-toxic and penetration enhancing effect of surfactant. This vascular system is gaining lots of interest due to its controlled and sustained action. This carrier system is having the immense opportunity in the area of transdermal delivery, cosmetics, neutraceuticals etc. Proniosomal gel has great drug delivery potential for cardiovascular drug, anticancer, anti-infective agents and in the pain management. In future, proniosomes might be experimented for more skin permeation and entrapment efficiency with optimized concentration of surfactant and other formulation parameters. Thus, proniosomes needs further research so as to bring out commercially available proniosomal preparation.

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