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Proniosomal Gel: A provesicular approach for transdermal drug delivery

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Abstract

Over the last few years a comprehensive research has been done over proniosomal gel as a provesicular approach for transdermal drug delivery. However skin has a very tough diffusion barrier that is lipid bilayer in the stratum corneum inhibiting penetration of drug moiety which is rate limiting barrier for penetration of drugs. Vesicular systems (niosomes & liposomes) are promising systems to cross this permeation barrier. They may act as vehicles or as permeation enhancer for bioactive materials to enhance their penetration via stratum corneum. But their major drawback is their unstability, which can be overcome by utilizing provesicular approaches like proniosomes. Proniosomes (gel) are semisolid liquid crystal products of nonionic surfactants easily prepared by dissolving the surfactant in a minimal amount of an acceptable solvent (ethanol) and the least amount of aqueous phase (water). Proniosomal gel offers a great potential to reduce the side effects of drugs and increased therapeutic effectiveness. Proniosomes can entrap both hydrophilic and hydrophobic drugs. In this review we have covered proniosomal drug delivery along with preparation, formulation and evaluation parameters of proniosomes as drug carrier.

Keywords: Niosome, Proniosomal gel, Nonionic surfactant, cholesterol, Phosphatidylcholine

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INTRODUCTION

Niosomes have received great attention as an alternative potential drug delivery system to conventional liposomes. Niosomes are uni or multilamellar spheroid structures composed of

amphiphilic molecules assembled into bi-layers. They are considered primitive cell models, cell-like bioreactors and matrices for bio-encapsulation. They are alternative to liposomes as they possess greater stability and overcome the problems associated with liposomes like chemical instability, variable purity of phospholipids and high cost [1]. The additional merits with niosomes are low toxicity due to non ionic nature, no requirement of special precautions and conditions for formulation and preparation [2]. Moreover the routine and the large-scale production of niosomes is very simple without the use of unacceptable solvent [3] and has great potential for controlled and targeted delivery of drugs [4]. It has been reported in several studies that compared to conventional dosage forms, vesicular formulations exhibited an enhanced cutaneous drug bioavailability [5]. The intercellular lipid barrier in the stratum corneum shows dramatically looser and more permeable property following treatment with liposomes and niosomes [2, 6,]. Both phospholipids and nonionic surfactants in the niosomes can act as penetration enhancers, which are useful for increasing the permeation of many drugs. Fusion of niosome vesicles to the surface of skin, results in higher flux of the drug due to direct transfer of drug from vesicles to the skin [6], but niosomes have certain limitations too such as physical instability, leakage of drug from vesicles on storage etc. So to increase the shelf life and stability of niosomes, proniosome are developed. Proniosomes changes to niosomes on hydration. Despite the strong rationale behind the applicability of vesicles (niosomes) in transdermal systems, the major problem in the development of vesicular systems at industrial and clinical levels is their somewhat unstable nature. Provesicular approach has been proposed to enhance the stability of vesicles. Proniosomes are provesicular approach which overcomes the limitations of vesicular system (Niosomes). Proniosomes can be converted into the niosomes in-situ by absorbing water from the skin.

Mechanism of drug transport through skin

As studies performed on the transdermal/topical application of vesicles have rendered conflicting results. It is still not clear which factors influence the vesicle–skin interactions and play an important role in determining the efficiency of drug transport through the skin. But it is clear that Proniosomes should be hydrated to form niosomal vesicles before the drug is released and permeates across the skin. Many scientists proposed different theories/mechanism for vesicle-skin interaction.

Two types of vesicle–skin interactions observed during in vitro studies using human skin which may induce various effects on dermal or transdermal drug delivery [7-9].

1. When vesicles come in contact with stratum corneum aggregate, fuse and adhere to the surface of cell. It is believed that this type of interaction leads to a high thermodynamic activity gradient of the drug at the interface of vesicle and stratum corneum, which is the driving force for penetration of the lipophilic drugs across the stratum corneum.
2. This type of interaction involves the ultra structural changes in the intercellular lipid regions of the skin and its deeper layers at maximum depth of about 10 mm as revealed by Freeze Fracture Electron Microscopy (FFEM) and Small Angle X-ray Scattering (SAXS).

In addition to these two several other mechanisms which could explain the ability of vesicles to modulate drug transfer across skin, including:

- Nature of drug

- The lipid bi-layers of niosomes act as a rate limiting membrane barrier for drugs
- Dehydration of vesicles
- The vesicles act as penetration enhancers to reduce the barrier properties of the skin
- Size and composition of vesicles
- Biophysical factors

Preparation of proniosomal gel

Coacervation phase separation method

This method is widely adopted to prepare proniosomal gel. Precisely weighed amounts of surfactant, lipid and drug are taken in a clean and dry wide mouth glass vial of 5.0 ml capacity and alcohol (0.5 ml) is added to it. After warming, all the ingredients are mixed well with a glass rod, the open end of the glass bottle is covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture is dissolved completely. Then the aqueous phase (0.1% glycerol solution) is added and warmed on a water bath till a clear solution was formed which is then converted into proniosomal gel on cooling [1, 10].

Formulation of proniosomal gel

Proniosomal gel is generally consisting of nonionic surfactant, cholesterol, lecithin, alcohol, aqueous phase and miscellaneous (Dicetyl Phosphate, Solulan C_{24} etc.)

Surfactants

Selection of surfactant should be done on the basis of HLB value. As Hydrophilic Lipophilic Balance (HLB) is a good indicator of the vesicle forming ability of any surfactant, HLB number in between 4 and 8 was found to be compatible with vesicle formation. It is also reported that the hydrophilic surfactant owing to high aqueous solubility on hydration do not reach a state of concentrated systems in order to allow free hydrated units to exist aggregates and coalesced to form lamellar structure. The water soluble detergent polysorbate 20 also forms niosomes in the presence of cholesterol. This is despite the fact that the HLB number of this compound is 16.7. Degree of entrapment is affected by the HLB of a surfactant. Transition temperature of surfactants also affects the entrapment of drug in vesicles. Spans with highest phase transition temperature provide the highest entrapment for the drug and vice versa [11, 12]. Span 40 and Span 60 produces vesicles of larger size with higher entrapment of drug. The drug leaching from the vesicles is reduced due to high phase transition temperature and low permeability. High HLB value of Span 40 and 60 results reduction in surface free energy which allows forming vesicles of larger size hence large area exposed to the dissolution medium and skin [13]. Different effects of non ionic surfactant on levonorgesterol permeation profile shows that the flux value is highest for Span 80 and lowest for Span 60. No significant difference is observed in the skin permeation profile of formulation containing Span 60 and Span 40 due to their higher phase transition temperature that is responsible for their lower permeability [1]. The encapsulation efficiency of Tween is relatively low as compared to Span.

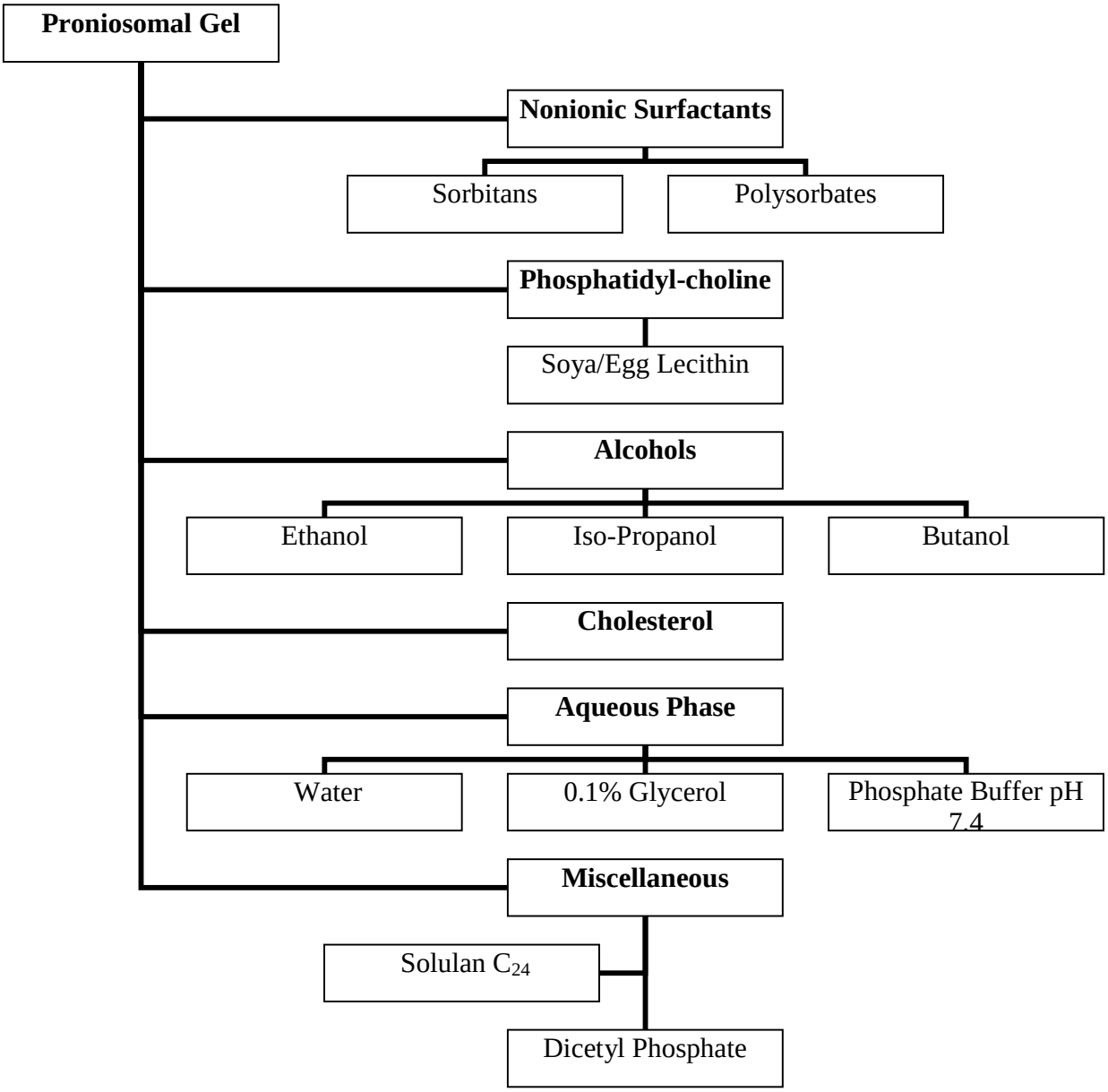

Fig. 1 Formulation of Proniosomal gel

Phosphatidyl choline

Phosphatidyl choline is such a major component of lecithin. Phosphatidyl choline has low solubility in water. In aqueous solution its phospholipids can form either liposomes, bilayer sheets, micelles or lamellar structures, depending on hydration and temperature. This results in a type of surfactant that is usually classified as amphipathic. They are a major component of biological membranes and can be easily obtained from a variety of readily available sources such as egg yolk or soy beans. Depending upon the Source from which they are obtained they are named as egg lecithin and soya lecithin. Incorporation of lecithin in proniosomes is justified as it acts as permeation enhancers. Incorporation of lecithin further enhanced the percent drug entrapment to 96.1% and leads to vesicles of smaller size due to increase in hydrophobicity which results in reduction of vesicle size [6]. There is probably formation of more compact and

well organized bi-layers which prevents the leakage of drug [15]. It is found that vesicle composed of soya lecithin are of larger size then vesicle composed of egg lecithin possibly due to difference in the intrinsic composition [1].

Table-1 Different Nonionic Surfactants Used in Preparation of Proniosomal Gel [14]

S.No.	Surfactant	Synonyms	Properties
1.	Sorbitan monolaurate	Span 20, Sorbitan monododecanoate	Tc : 16ºC Density: 1.032 g/mL at 25°C (lit.) Flash point:>230°F HLB value: 8.6
2.	Sorbitan monopalmitate	Span 40,	Tc : 42ºC Flash point:113ºC Melting point: 46-47ºC HLB value: 6.7
3.	Sorbitan monostearate	Span 60, Sorbitan monooctadecanoate	Tc : 53ºC Flash point: >110ºC Melting point: 54-57ºC HLB value: 4.7
4.	Sorbitan monooleate	Span 80, Sorbitan (Z)-mono-9-octadecenoate	Tc : -12ºC Flash point: >110ºC Density: 0.986 HLB value:4.3
5.	Polyoxyethylene (20)sorbitan monolaurate	Tween 20	Density: 1.106 Aq.solubility: 100 g/L Boiling point: 100 ºC HLB value: 16.7
6.	Polyoxyethylene (20)sorbitan monopalmitate	Tween 40	Density: 1.05 Aq.solubility: 100 g/L Boiling point: 0.1 ºC HLB value: 15.6
7.	Polyoxyethylene (20)sorbitan monostearate	Tween 60	Density: 1.081 Aq.solubility: 100 g/L HLB value: 14.9
8.	Polyoxyethylene (20)sorbitan monooleate	Tween 80	Density: 1.064 Aq.solubility: 5-10 g/100 mL at 23 ºC Flash point: >110 ºC HLB value: 15.0

Tc ─ Phase transition temperature
HLB ─ Hydrophilic Lipophilic Balance

Cholesterol

Cholesterol is essential component of vesicles. Incorporation of cholesterol influence vesicle stability and permeability [16]. Concentration of cholesterol plays an important role in

entrapment of drug in vesicles. There are reports that entrapment efficiency increase with increasing cholesterol content and by the usage of span 60 which has higher transition temperature. It was also observed that very high cholesterol content had a lowering effect on drug entrapment to the vesicles. This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bi-layered structure leading to loss of drug entrapment.

Solvent

Alcohol used in Proniosomes has a great effect on vesicle size and drug permeation rate [1]. Vesicles formed from different alcohols are of different size and they follow the order: Ethanol > Propanol > Butanol > Isopropanol. Highest size of vesicle in case of ethanol is due to its greater solubility in water and smallest size of isopropanol, may be due to branched chain present in it.

Aqueous Phase

Phosphate buffer pH 7.4[5], 0.1% glycerol [10], hot water [18] are used as aqueous phase in Preparation of proniosomal gel.

Miscellenous

Dicetyl Phosphate (DCP)

Dicetyl phosphate is a charged lipid induces negative charge to vesicles. Proniosomal formulation containing DCP incorporate slightly greater amount of drug as compare to formulation containing surfactant and cholesterol only but much less than those formulation containing egg/soya lecithin. It is also reported that drug release was maximum for the formulation containing DCP perhaps due to the charge present in the DCP containing bi-layers, which is responsible for increase in the curvature and decrease vesicle size [1]. DCP decreases the entrapment efficiency of drug into niosomal vesicle [18].

Stearyl amine (SA)

Stearyl amine is also a charged lipid used to impart positive charges on niosomal vesicles. It is reported that SA decreases the entrapment efficiency.

Solulan

Solulan C_{24} (poly-24 oxyethylene cholesteryl ether) must be added to the formulation to ensure a homogenous formulation devoid of aggregates.

Characterization of proniosome derived niosomes

Vesicle diameter

Niosomes are spherical in shape and their diameter can be determined by using light microscope, photon correlation spectroscopy, freeze-fracture electron microscopy, SEM and TEM.

Entrapment efficiency

After preparation of niosomes, the entrapped drug is separated by dialysis, centrifugation, gel chromatography or filtration. The drug encapsulated in niosomes is determined by complete destruction of vesicles using 50% propane or 0.1% triton x 100 or un-entrapped drug can be subtracted from total amount of drug.

Table-2 Drugs Encapsulated in Proniosomal Gel [1, 2, 10, 15, 18-22]

Sr. No.	Name of the active agent	Pharmacological Class	Investigator (Year)
1.	Levonorgesterol	Contraceptive agent	Vora et al (1998)
2.	Estradiol	Hormone	Fang et al (2001)
3.	Ketorolac	NSAID'S	Ibharim et al (2005)
4.	Chlorpheniramine Maleate	Skin disorders	Varshosaz et al (2005)
5.	Captopril	Antihypertensive	Gupta et al (2007)
6.	Flurbiprofen	NSAID'S	Mokhtar et al (2008)
7.	Hydrocortisone	Corticosteroid hormone	Shanker et al (2009)
8.	Losartan Potassium	Angiotensin ll antagonist	Thakur et al (2009)
9.	Furosemide	Antihypertensive	Azeem et al (2009)

Table- 3 Different methods for separation of the entrapped and un-entrapped drug [17]

Separation method	Advantages	Disadvantages
Exhaustive dialysis	Suitable for large vesicles > 10 µm suitable for highly viscous systems, inexpensive	Extremely slow (5-24 h) Large volumes of dialysate required- (may not be suitable for drugs requiring specialized disposal) Dilutes the niosome dispersion
Centrifugation (below 7000 × g)	Quick (~30 min) Inexpensive instrumentation Concentrates the niosome dispersion	Fails to sediment the sub-micron dispersion May lead to the destruction of fragile systems
Ultracentrifugation (150 000 × g)	Sediments all size populations Concentrates the noisome dispersion	Expensive instrumentation Long centrifugation times (1-1.5h) May lead to the destruction of fragile systems
Gel filtration	Quick (4-5 min with sephadex g50)	Slow (1-2h when using sepahrose 2b/4b for macromolecule separation) Gels are expensive if not reused Dilutes the noisome dispersion Not suitable for highly viscous formulations

The methods that have been used for the removal of un-entrapped material include:

1. Exhaustive dialysis
2. Gel filtration
3. Centrifugation
4. Ultra centrifugation

The entrapment efficiency is expressed by the following formula.

$$\text{Entrapment efficiency} = \frac{\text{Amount entrapped}}{\text{Total amount of drug}} \times 100$$

Rate of hydration (Spontaneity)

Spontaneity of niosomes formation is described as number of niosomes formed after hydration of proniosomes for 15 min. Proniosomes were transferred to the bottom of a small stoppered glass tube and spread uniformly. One ml saline (0.154 M NaCl) was added carefully along the walls of the test tube and kept aside without agitation. After 15-20 min a drop of aqueous layer was withdrawn and placed on Neubaur's chamber. The number of niosomes eluted from proniosomes was counted.

Spontaneity studies showed that niosomes containing isopropanol and butanol were formed more spontaneously than niosomes containing propanol and ethanol perhaps due to faster phase separation of isopropanol and butanol due to their lower solubility in water [1, 10].

Zeta Potential

Zeta potential is a measure of net charge on surface of niosomes. Lower the charge on the surface of noisome lower the repulsive force between the vesicles. Due to low repulsive forces agglomeration occurs which provide unevenly distributed suspension, faster rate of settling results in unstable niosomal suspension.

***In vitro* release**

In vitro release can be determined by dialyzing the proniosomal gel against buffer/specified media at definite temperature and determining the content of dialysate. Different methods are specified in literature to determine in vitro release are as follows:-

- Proniosomal gel was spread on glass circular disk (5.04 cm diameter), then covered by cellophane dialyzing membrane with molecular weight cut-off of 8000 which was securely mounted on the disk by a rubber band. The disk was placed on the bottom of a glass tube large enough to accommodate the disk diameter and 50 ml of dialysate was poured onto the membrane surface. The whole assembly was immersed in a water bath maintained at 37.8^{o}C. The buffer solution was continuously circulated over the membrane surface in a closed circle at a rate of 5 ml/min using a Watson–Marlow peristaltic pump [3].
- In vitro release studies on proniosomal gel were performed using locally manufactured Franz-diffusion cell. The dialysis cellophane membrane was mounted between the donor and receptor compartment. A weighed amount of proniosomal gel was placed on one side

of the dialysis membrane. The receptor medium was phosphate saline buffer pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1^{o}C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a Teflon coated magnetic bead fitted to a magnetic stirrer. At each sampling interval, samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion were analyzed [10].

***In vitro* permeation study**

- The permeation of drug from proniosome formulation are determined by using Franz (vertical) diffusion cell. The wistar rat (7–9 weeks old) skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The top of the diffusion cell was covered with paraffin paper. The donor compartment was filled with the proniosome formulation. The receptor compartment was maintained at 37°C and stirred by a magnetic bar at 600 rpm. At appropriate intervals, 200 µl aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The samples were analyzed by HPLC/UV method [10].
- Permeation study of proniosomal gel can be done by using Keshary-Chien type diffusion cell. The proniosomal formulation was adhered to the furry side of the skin. This formulation applied skin was mounted and clamped between the donor and receptor compartment with furry side facing upward (donor side). The receptor compartment was surrounded by a water jacket for maintaining the temperature at 37'C. The temperature was maintained using a thermostatic hot plate temperature control available on the magnetic stirrer. The receptor fluid was stirred by magnetic bead operated on a magnetic stirrer. The top of the donor compartment was open for air circulation. At each sampling interval of 1 hour samples were withdrawn from sampling port and were replaced with same volume of the fresh receptor fluid every time. Samples withdrawn were analyzed by an appropriate method [1].

Stability studies

Stability of a formulated product on shelf is an important factor in successful development of a dosage form. Very few reports are available on shelf storage of niosomal preparations. The stability studies of prepared niosomes are performed at accelerated conditions of humidity and temperature and drug content is noted using suitable Technique (UV/HPLC).

CONCLUSION

These days vesicular systems have been gaining a lot of interest of various researchers and scholars. It is because of their advantages of controlled and sustained release action, stability and versatility as a drug carrier. These carrier systems have immense scope in future, especially in the area of transdermal drug delivery, eg. In dermatitis, periodontitis, cosmetics etc. The other future area which can be focused for research might be some other types of new proniosomes with more pronounced entrapment efficiency and skin permeability by trying various ingredients in optimized concentration.

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