AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Proniosomes: A Recent Advancement In Nanotechnology As A Absorption Modulator for Transdermal Drug Delivery System

Dinesh Chandra*[1], Ashish Kumar[1], Rakesh singh[1], Tirth Raj[1], Shrikant Verma[2], Niraj Sharma

1.Kamla Nehru Institute of Management and Technology, Sultanpur, India.
2.Babu Banarasi Das University, Lucknow, India.
3.Dr.K.N. Modi Institute of Pharmaceutical Education & Research, Modinagar, India

ABSTRACT

The proniosomal approach helps to solve the problem regarding stability and provides higher entrapment efficiency over conventional system. Proniosomal gel is a liquid crystalline- compact niosomal hybrid which is prepared by dissolving surfactant in small amount of suitable solvent and least amount of aqueous phase. This compact gel can be converted to niosomes hydration. 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 rout. Over the last few years an inclusive research has been done over pro-vesicular approach for transdermal drug delivery. Skin has a very tough diffusion barrier inhibiting penetration of drug moiety which is rate limiting barrier for penetration of drugs. There are several approaches that deal with penetration enhancement across the skin. Vesicular and provesicular systems are promising amongst them. Vesicular systems including (niosomes, ethosomes, transfersomes and liposomes) are promising systems to cross this permeation barrier.

Keywords: Proniosomes, Niosomes, skin penetration, stability, transdermal, coacervation.

*Corresponding Author Email: ashishkumar8081405109@gmail.com
Received 02 November 2013, Accepted 19 November 2013

Please cite this article in press as: Ashish *et al* Proniosomes: A Recent Advancement In Nanotechnology As A Absorption Modulator for Transdermal Drug Delivery System , India. American Journal of PharmTech Research 2013.

INTRODUCTION

Colloidal particulate carriers such as liposomes or niosomes have been widely employed in drug delivery systems and producing them from proniosomes provides them a distinctive advantage. These carriers can act as drug reservoirs and the rate of drug release can be controlled by modification of their composition[1,2,3,4]. Human skin is the important target site for the application of drug especially in the treatment of local disease. Penetration enhancement with special formulation approaches is mainly based on the usage of colloidal carriers[5,6]. Drug encapsulated in lipid vesicles prepared from phospholipids and nonionic surfactant is known to be transported into and across the skin. Because of their ability to carry a variety of drugs, liposomes have been extensively investigated for their potential application in pharmaceutics; such as drug delivery for drug targeting; for controlled release or for increasing solubility[7,8,9,10]. No doubt that drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have proved to possess distinct advantages over conventional dosage forms because the particles can act as drug reservoirs, can carry both hydrophilic drugs by encapsulation or hydrophobic drugs by partitioning of these drugs into hydrophobic domains and modification of the particle composition or surface can adjust the drug release rate and/or the affinity for the target site. The vesicles in a dispersed aqueous system may suffer from some chemical problems associated with degradation by hydrolysis or oxidation as well as physical problems as sedimentation, aggregation, or fusion of liposomes during storage[11]. Liposomes also have been the potential of overcoming the skin barrier, as these are bilayered lipid vesicles, consisting primarily of phospholipids and cholesterols[12]. Vesicular systems have been widely studied as vehicles for dermal and transdermal drug delivery. Their benefits in enhancing drug permeation have been well established[13]. Amongst the liposomes, transferosomes, ethosomes and niosomes have been reported to offer enhanced permeability through stratum corneum barriers.

Transdermal route*:*

Transdermal is a route of administration wherein active ingredients are delivered across the skin for systemic distribution. Transdermal drug delivery is an alternative to the conventional oral and parental routes as a means of achieving constant therapeutic levels of the drug. Proniosomes offer a versatile vesicle drug delivery concept with potential for delivery of drugs via transdermal route. This would be possible if proniosomes form niosomes upon hydration with water from skin following topical application under occlusive conditions[14].

PRONIOSOMES:[15]

The advancements in the niosome lead to the evolution of proniosomal delivery systems.

Proniosomes are non-ionic based surfactant vesicles which may be hydrated immediately before use to yield aqueous niosome dispersions. Proniosomes are nowadays used to enhance drug delivery in addition to conventional niosomes. They are converted into niosomes respectively upon simple hydration or by the hydration of skin itself after application.

Proniosomes exist in two forms

i) Semisolid liquid crystal gel.

ii) Dry granular powder

Of these two forms the proniosomal gel is mainly used for topical / transdermal application.

Advantage of Proniosomes

- Proniosomes required no special condition during their preparation storage as in the case of liposomes and niosomes[16].
- Minimizes the problems of physical instability of niosomes on storage.
- In aqueous suspension liposomes have problems regarding degradation by oxidation and hydrolysis of phospholipids molecules.
- Ease to transfer, distribution, measuring, and storage.
- Proniosome is dry product which could be easily hydrated immediately before use i.e easy to use[17].
- Proniosome is free flowing powder uniform in size.
- Cheap as compared to noisome.
- Exhibit better purity than liposomes.
- Hydration is much easier than the long shaking process requiring in the case of liposome and niosomes.
- Proniosome are dry powder, which makes further processing and packaging possible.
- The powder form provides optimal in capsules could be beneficial.
- Avoiding the problem of physical stability like fusion, aggregation, sedimentation and leakage on storage[18] .
- Drug delivery with improved bioavailability, reduced side effects[19].

Niosomes:

Handling and storage of surfactants require no special conditions. Niosomes possess an infrastructure consisting of hydrophilic and hydrophobic moieties together, and as a result, can accommodate drug molecules with a wide range of solubilities[20]**.** The size of niosomes are microscopic and lies in nanometric scale. The particle size ranges from 10nm-100nm. Niosomes are non-ionic surfactant vesicles that can entrap a solute in a manner analogous to liposomes.

They are osmotically active, and are stable on their own, while also increasing the stability of the entrapped drugs[21,22]. Although niosomes as drug carriers have shown advantages such as being cheap and chemically stable, they are associated with problems related to physical stability such as fusion, aggregation, sedimentation and leakage on storage[23].

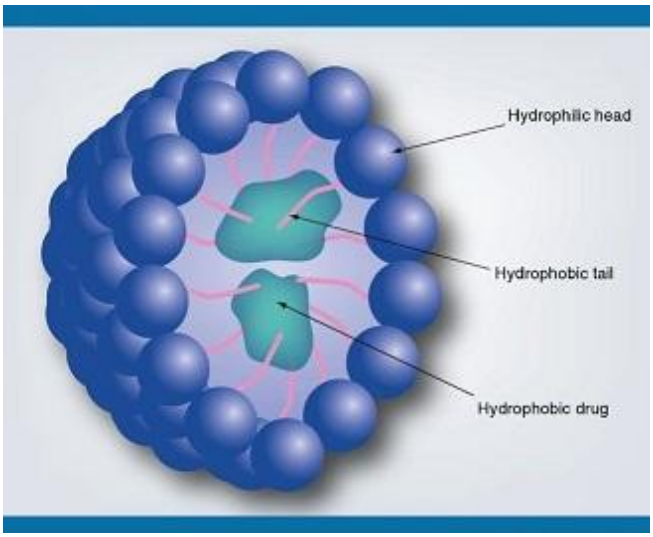

Figure 1: Representation of Niosomes.

Disadvantages of Niosomes:

1. Physical instability
2. Aggregation
3. Fusion
4. Leaking of entrapped drug
5. Hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion

Advantages of proniosomes over the niosomes[24-27]:

- ❖ Avoiding problem of physical stability like aggregation, fusion, leaking.
- ❖ Avoiding hydrolysis of encapsulated drugs which limiting the shelf life of the dispersion.

MATERIALS AND METHOD

Non ionic surfactants

A wide range of surfactants are available and theselection of surfactants should be done on the basis of Hydrophilic Lipophilic balance. The HLB in between 4 to 8 was found to be compatible with vesicles formulations[33]. 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. The encapsulation efficiency of tween is low as compared to span because of the larger size of vesicles and less lipophilic nature of tween. When span is used it also increases the lipophilicity of drug[34]. Degree of entrapment is affected by HLB number.

Transition temperature of surfactants also affects entrapment of drug in vesicles. Spans have highest phase transition temperature provides the highest entrapment for the drug[35].

Table I: Materials and methods of proniosomes.

Sr. No.	Ingredients	Example	Use	Ref(s)
1.	Surfactant (Non-ionic)	Tween (20, 40, 60, 80), span (20, 40, 60),Brij 10,35,72	To increase rate of permeation	28
2.	Cholesterol	Cholesterol	To improve stability of vesicle	29,30
3.	Lecithin	Soya and egg lecithin	Penetration enhancer	31
4.	Solvents	Ethanol , Methanol	For solubilising drug, Surfactant	32
5.	Aqueous phase	Hot water, buffer, glycerol	Entrapment efficiency	32

Lecithin

Incorporation of lecithin in proniosomes may act as permeation enhancer, prevents the leakage of drug and enhanced the percent drug entrapment due to high phase transition temperature. The vesicles composed of soya lecithin are of larger size then vesicle composed of egg lecithin due to difference in the intrinsic components. On the basis of penetration capability the soya lecithin is considered as a good candidate as it contains unsaturated fatty acids, oleic and linoleic acid while egg lecithin contains fatty acids[36]. Phosphotidyl choline is the major component of lecithin. The name basically depends upon their source of origin such as soya lecithin from soya beans and egg lecithin from egg yolk. Phosphotidyl choline has low solubility in water[37].

Cholesterol

Concentration of cholesterol plays an important role in entrapment of drug in vesicles. The entrapment efficiency and permeation increases with increasing cholesterol content and by the usage of span 60 which has higher transition temperature[38]. Cholesterol is an essential component of vesicles. Incorporation of cholesterol influence vesicles stability and permeability[39]. On further increase in cholesterol beyond certain concentration level starts disrupting the regular bi-layered structure leading to loss of drug entrapment and permeation[40].

Solvent

Vesicles formed from different alcohols are of different sizes and they follow the order: ethanol > propanol > butanol > isopropanol. Higher size of vesicles in case of ethanol is due to its greater solubility in water and smallest size of isopropanolol, may be due to branched chain present in it[41]. Selection of solvent is another important aspect as it has great effect on vesicle size and drug permeation rate[42]. Ethanol may cause the reduction of lipid polar head interactions within the

membrane, thereby increased the skin permeation[43,44].

Aqueous phase

Phosphate buffer 7.4, 0.1% glycerol and hot water are mainly used aqueous phase for proniosomes. pH of the hydrating medium also play important role in entrapment efficiency. The aqueous medium might influence the tactness of proniosomes, thus affecting their entrapment efficiency[45,46].

Methodology

Preparation of proniosomes:

The proniosomes can be prepared by

- Slurry method.
- Slow spray coating method.
- Coacervation phase separation method.

Slurry method

Proniosomes can be prepared from a stock solution of surfactants and cholesterol in suitable solvent. The required volume of surfactant and cholesterol stock solution per gram of carrier and drug should be dissolved in the solvent in 100 ml 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 Hg until the mass in the flask had become a dry, free flowing product. Finally, the formulation should be stored in tightly closed container under refrigeration in light[47].

Slow spray coating method

A 100 ml round bottom flask containing desired amount of carrier can be attached to 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[47].

Coacervation phase separation method

Proniosomal gels can be prepared by this method which comprises of surfactant, lipid and drug in a wide mouthed glass vial along with small amount of alcohol in it. The mixture is warmed over water bath at 60-700C for 5min until the surfactant mixture is dissolved completely. Then

the aqueous phase is added to the above vial and warmed still a clear solution is formed which is then converted into proniosomal gel on cooling[78,79]. After hydration of proniosomes they are converted to uniformly sized niosomes.

Formation of Niosomes from Proniosomes[49] :

The niosomes can be prepared from the proniosomes by adding the aqueous phase with the drug to the proniosomes with brief agitation at a temperature greater than the mean transition phase temperature of the surfactant.

$$T > Tm$$

where,

T = Temperature

Tm = mean phase transition temperature

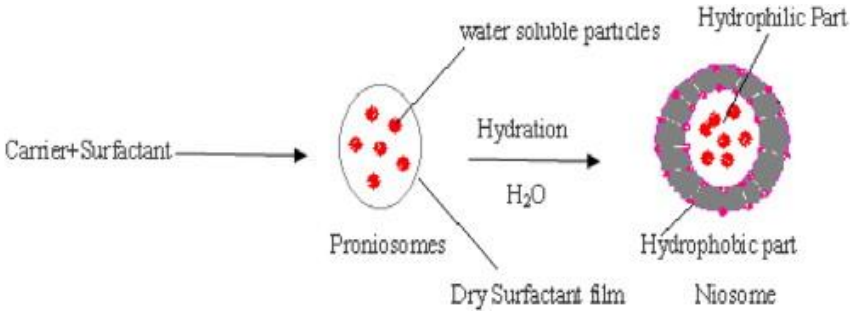

Figure 2: Formation of Niosomes from Proniosomes.

CHARACTERIZATION OF PRONIOSOMES[47]

Various methods for characterization of proniosomes are shown in Table II.

Table II: Methods of Characterization of Proniosomes

S.No.	Parameter(s)	Method(s)
1.	Vesicle size analysis	Scanning electron microscopy, transmission electron microscopy, laser diffraction method
2.	Entrapment efficiency	Diode array spectrophotometer, centrifugation method, dialysis method
3.	*In- vitro* permeation study	Franz diffusion cell, Flow through diffusion cell, Keshary chien diffusion cell.
4.	*In- vitro* release study	Dialysis bag method
5.	Zeta potential determination	Zeta potential probe model

Vesicle size and morphology

Vesicle morphology involves the measurement of size and shape. The size of the vesicles can be measured by light scattering method and optical microscopy in two conditions *i.e:* with agitation

and without agitation. Hydration without agitation results in largest vesicle size. Surface morphology means roundness, smoothness and formation of aggregation; it can be studied by scanning electron microscopy and transmission electron microscopy[50,51].

Scanning electron microscopy

Particle size of proniosomes is very important characteristic. The surface morphology (roundness, smoothness, and formation of aggregates) and the size distribution of proniosomes were studied by Scanning Electron Microscopy (SEM). For scanning electron microscopy, the niosomes are mounted on an aluminum stub using double sided adhesive carbon tape. Then the vesicles are sputter coated with gold palladium (Au/Pd) using a vaccum evaporator and examined using a Scanning electron microscopy equipped with a digital camera at 25kV accelerating voltage[52,53].

Transmission electron microscopy

The morphology of hydrated niosome dispersion prepared from proniosome was also determined using transmission electron microscopy (TEM). The noisome dispersion is applied to a carbon-coated 300 mesh copper grid and left to allow some of the niosomes to adhere to the carbon substrate. The remaining dispersion is removed by absorbing the drop with the corner of a piece of filter paper. Then drop of aqueous solution of uranyl acetate is applied. The remaining solution is removed by absorbing the liquid with the tip of a piece of filter paper and the sample is air dried and observed under transmission electron microscope[54,55].

Entrapment efficiency

Various methods can be used to evaluate the loading capacity of proniosomal systems such as dialysis method, gel filtration and centrifugation method[56,57]. In dialysis method, amount of entrapped drug can be obtained by subtracting the amount of untrapped drug from total drug incorporated.

$$\text{Entrapment efficiency(EE)} = \text{Amount of drug entrapped/total amount of drug} \times 100$$

In centrifugation method , centrifugation should be used at 1400 rpm for 40 minutes at 40°C and entrapment efficiency can be calculated as

Above[58,59].

***In-vitro* Drug Release From Proniosomal Vesicles**:

In-vitro drug release and skin permeation studies for proniosome scan be determined by different techniques like

- Franz diffusion cell

- Dialysis tubing
- Reverse dialysis

Franz Diffusion Cell:

This franz diffusion cell has a donor chamber fitted with a cellophane membrane. The Proniosomes are placed in it and dialyzed against a suitable dissolution medium at room temperature. The drug content is analyzed using suitable method (UV spectroscopy, HPLC) maintenance of sink conditions is essential[60].

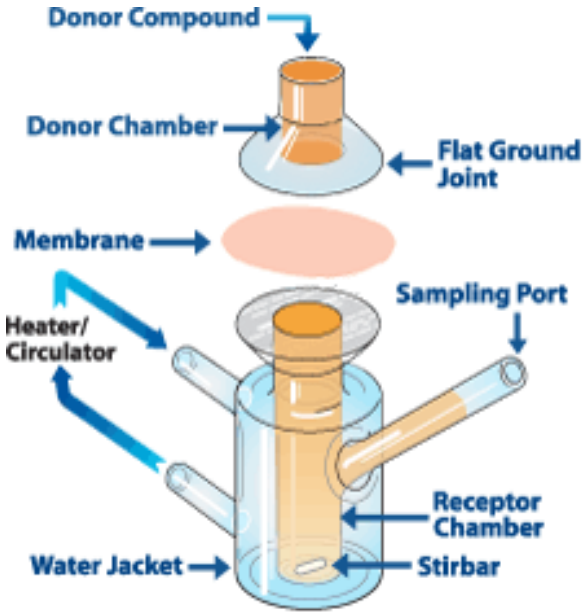

Figure 3: Franz Diffusion cell

Dialysis tubing[77]:

Muller et al in 2002 studied in vitro drug release could be achieved by using dialysis tubing. The proniosomes is placed in prewashed dialysis tubing which can be hermetically sealed. The dialysis sac is then dialyzed against a suitable dissolution medium at room temperature; the samples are withdrawn from the medium at suitable intervals, centrifuged and analysed for drug content using suitable method (U.V. spectroscopy, HPLC etc). The maintenance of sink condition is essential.

Reverse dialysis[77]:

In this technique a number of small dialysis as containing 1ml of dissolution medium are placed in proniosomes. The proniosomes are then displaced into the dissolution medium. The direct dilution of the proniosomes is possible with this method; however the rapid release cannot be quantified using this method.

Zeta potential determination

Zeta potential can be analyzed to measure the stability of niosomes by studying its colloidal property. The zeta potential of indomethacin proniosomes was measured by a zeta potential probe. Zeta potential analysis is a measure of net charge of niosomes. The higher charge on the surface of vesicles produce repulsive force between the vesicles which made them stable, devoid of agglomeration and faster settling, providing an evenly distributed suspension[61].

Marketed Products:

Lancôme has come out with a variety of antiageing products which are based on noisome formulations. L'Oreal is also conducting research on anti-ageing cosmetic products. Niosomal Preparation in the Market is **– Lancôme** (www.lancome.com)

APPLICATION OF PRONIOSOMES

(1) Drug targeting:

One of the most useful aspects of proniosomes is their ability to target drugs. Proniosomes can be used to target drugs to the reticulo-endothelial system. The reticulo-endothelium system[62]. (RES) preferentially takes up proniosomes vesicles. The uptake of proniosomes is controlled by circulating serum factors called opsonins. These opsonins mark the noisome for clearance. Such localization of drugs is utilized to treat tumors in animals known to metastasize to the liver and spleen. This localization of the drugs can also be used for treating parasitic infections of the liver. Proniosomes can also be utilized for targeting drugs to organs other than the RES. A carrier system (such as antibodies) can be attached to proniosomes (as immunoglobin bind readily to the lipid surface of the noisome) to target them to specific organs[63].

(2) Anti-neoplatic treatment:

Most antineoplastic drugs cause severe side effects. Proniosomes can alter the metabolism; prolong circulation and half life of the drug, thus decreasing the side effects of the drugs. Proniosomal entrapment of Doxorubicin and Methotrexate[64,65], (in two separate studies) showed beneficial effects over the unentraped drugs, such as decreased rate of proliferation of the tumor and higher plasma levels accompanied by slower elimination[66].

(3) Treatment of leishmaniasis[67]:

Leishmanasis is a disease in which a parasite of the genus Leishmania invades the cells of the liver and spleen. Commonly prescribed drugs for the treatment are derivatives of antimony (antimonials), which in higher concentrations can cause cardiac, liver and kidney damage. Use of proniosomes in tests conducted showed that it was possible to administer higher levels of the drug without the triggering of the side effects, and thus allowed greater efficacy in treatment.

(4) Delivery of peptide drugs[68]:

Oral peptide drug delivery has long been faced with a challenge of bypassing the enzymes which would breakdown the peptide. Use of proniosomes to successfully protect the peptides from gastrointestinal peptide breakdown is being investigated. In an in-vitro study, oral delivery of a Vasopressin derivative entrapped in proniosomes showed that entrapment of the drug significantly increased the stability of the peptide.

(5) Uses in studying immune response[69]:

Proniosomes are used in studying immune response due to their immunological selectivity, low toxicity and greater stability. Proniosome are being used to study the nature of the immune response provoked by antigens.

(6) Niosomes as carriers for haemoglobin[70]:

Proniosomes can be used as carriers for haemoglobin within the blood. The proniosomal vesicle is permeable to oxygen and hence can act as a carrier for haemoglobin in anaemic patients.

(7) Transdermal drug delivery systems[71]:

One of the most useful aspects of proniosomes is that they greatly enhance the uptake of drugs through the skin. Transdermal drug delivery utilizing proniosomal technology is widely used in cosmetics; In fact, it was one of the first uses of the niosomes[72]. Topical use of proniosome entrapped antibiotics to treat acne is done. The penetration of the drugs through the skin is greatly increased as compared to un-entrapped drug. Recently, transdermal vaccines utilizing proniosomal technology is also being researched. The proniosome (along with liposomes and transferomes) can be utilized for topical immunization using tetanus toxoid. However, the current technology in proniosomes allows only a weak immune response, and thus more research to be done in this field.

(8) Sustained release[73]:

The role of liver as a depot for methotrexate after proniosomes are taken up by the liver cells. Sustained release action of proniosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in thecirculation via proniosomal encapsulation.

(9) Localized drug action[74]:

Drug delivery through proniosomes is one of the approaches to achieve localized drug action, since their size and low penetrability through epithelium and connective tissue keeps the drug localized at the site of administration. Localized drug action results in enhancement of efficacy of potency of the drug and at the same time reduces its systemic toxic effects e.g. Antimonials encapsulated within proniosomes are taken up by mononuclear cells resulting in localization of

drug, increase in potency and hence decrease both in dose and toxicity. The evolution of proniosomal drug delivery technology is still at an infancy stage, but this type of drug delivery system has promise in cancer chemotherapy and anti-leishmanial therapy.

Different studies related to application of proniosomes as a carrier system[75,76]

Table III: Proniosomes as a carrier of various drug molecules has been cited in Table.

S. No	Drug	Hydrophilic Or Lipophilic	Category	Result(s)
1.	Ibuprofen	Lipophilic	NSAIDS	Proniosomes derived niosomes are superior in their ability to release the drug at a constant rate.
2.	Aceclofenac	Lipophilic	NSAIDS	The polynomial equation and mcontour plots developed by using central composite design allowed to prepare Proniosomes with optimum characteristic.
3.	Haloperidol	Hydrophilic	Antipsychotic effect	The formulation with single surfactant increased the permeation of drug more than those with mixture of surfactants
4.	Piroxicam	Lipophilic	NSAIDS	Span 60 based lecithin vesicle showed significant decrease in paw swelling. There is a increased drug delivery from lipid vesicles.
5.	Indomethacin	Lipophilic	NSAIDS	The release rate of the drug from the vesicle was in the controlled manner.
6.	Alprenolol hydrochloride	Lipophilic	Antihypertensive	The use of the maltodextrin in Proniosomes helps in enhancement of drug release.
7.	Captopril	Hydrophilic	Antihypertensive	Prolonged release of captopril
8.	Griseofulvin	Lipophilic	Antifungal	Enhanced absorption of the drug
9.	Flurbiprofen	Lipophilic	NSAIDS	The drug release rate from cholesterol free proniosomes was found to be high.
10.	Estradiol	Lipophilic	For symptomatic Treatment of the usual symptoms associated with menopause	The non ionic surfactant in Proniosomal formulation helps in enhancement of drug permeation through the skin.
11.	Ketorolac	Lipophilic	NSAIDS	The drug entrapment was high within the lipid bilayers of vesicles
12.	Losartan potassium	Hydrophilic	Antihyper tension	Enhanced bioavailability & skin permeation of drug
13.	Levonorgestrel	Lipophilic	Anticontra cepti-ves	The study demonstrated the utility of proniosomal transdermal patch bearing levonorgestrel for effective contraception
14.	Celecoxib	Lipophilic	Cyclooxy genase - inhibitor	Enhanced bioavailability of Celecoxib
15.	Cromolyn Sodium	Hydrophilic	Antiasthmatic and antiallergic	High nebulisation efficiency percentage and good physical stability were observed.

CONCLUSION

They have emerged as a challenging carriers for drug delivery via transdermal/topical route. It has become a useful dosage form for transdermal drug delivery due to the simple and cost effective scale up production procedure. The system had attracted researchers as an alternate strategy for transdermal delivery of drugs because it reduces the toxicity and enhances penetration effect of surfactants. These carrier systems have immense scope in future, especially in the area of transdermal drug delivery. Proniosome are very promising drug carriers as compared to liposome and noisome. Compared to liposome and noisome suspension, proniosome represent a significant improvement by eliminating physical stability problems, such as aggregation of fusion of vesicles and leaking of entrapped drug during long term storage.

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