

Proniosomes for Penetration Enhancement in Transdermal System

Samita Singla, S. L. HariKumar and Geeta Aggarwal

Rayat and Bahra Institute of Pharmacy, Sahauran, Kharar, District Mohali, Punjab, India-140104.

Abstract

Over the last few years an inclusive research has been done over 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 vesicular systems are promising amongst them. Vesicular systems including (niosomes, ethosomes, transfersomes and liposomes) are promising systems to cross this permeation barrier. But their major drawback is their unstability, which can be overcome by using vesicular approaches like proniosomes, protransfersomes and proliposomes. Vesicular approaches have been proposed to enhance the stability of vesicles. These approaches may overcome skin barrier properties and enhance percutaneous absorption. In these systems both type of drugs, hydrophilic and lipophilic drugs can be incorporated. The focus of this review is to bring together different aspects related to mechanism of skin permeation of vesicular system, proniosomes preparation and its applications as a carrier system.

Key words:

Vesicular system, proniosomes, skin penetration, stability, transdermal.

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Introduction

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^[1,2]. Colloidal carriers have attracted the main interest because they are promising systems having localized effect. These carrier, accumulate in stratum corneum or other

*Corresponding author, Mailing address:
Dr. Geeta Aggarwal (Assistant Professor)
Rayat and Bahra Institute of Pharmacy,
Sahauran, District Mohali, Punjab -140104, India
E.mail: geeta285@yahoo.co.in

upper skin layers are not expected to penetrate into viable skin^[3]. 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^[4]. 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^[5,6]. A number of vesicles systems such as liposomes, niosomes, ethosomes, emulsomes and transfersomes have been developed. The vesicular carrier such as niosomes has distinct advantage over conventional dosage forms because these particles can act as drug reservoir^[7]. Niosomes are non ionic surfactant based multilamellar or unilamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulted from organization of surfactants macro molecules as bilayers^[8,9]. The non ionic surfactants for this use are usually single alkylchain surfactant or sorbitan esters. They are biodegradable, biocompatible and non immunogenic in nature. Niosomes have potential applications in the delivery of hydrophobic as well as hydrophilic drugs^[10]. These vesicles are analogous to the liposomes (phospholipid vesicles) and serve as drug carrier because they can encapsulate both hydrophilic and lipophilic substrates^[11]. Compared with the liposomes, niosomes offers higher osmotic stability with lower cost and greater availability of surfactants^[12,13]. They improve the therapeutic performance of the drug molecules by delayed clearance from the circulation,

protecting the drug from biological environment and restricting effects to target cells^[14]. They have some disadvantages like physical and chemical instability, aggregation, fusion of vesicles and leaking or hydrolysis of the encapsulated drug. These factors may contribute in decreasing niosomal shelf life^[15,16]. The disadvantages of vesicular system have been shown in Figure 1. To overcome these problems a noble provesicular approach proniosomes were developed. 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^[17]. Proniosomal system serves as a rate limiting barrier for absorption of drugs. These systems can overcome the permeation barrier of the skin and act as a penetration enhancers for the drugs^[18]. The vesicles may serve as non toxic penetration enhancer for drug because of the amphiphilic nature of the vesicles; they are more stable and compatible with the skin^[19]. Provesicular system can be simply converted into vesicular system, which presents a useful vesicular delivery concept with potential to deliver drugs via transdermal route.

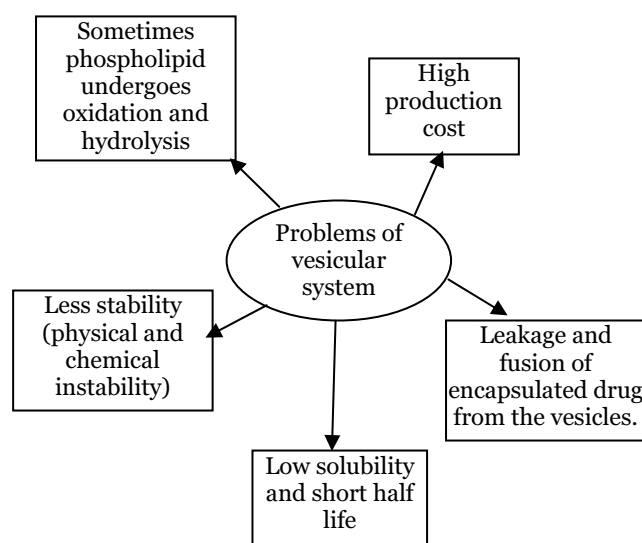


Figure 1: Problems of vesicular system.

Advantages of proniosomes

Potential applications of proniosomes includes improvement in bioavailability and permeation of the drug, enhances the skin permeation and develop a transdermal therapeutic system, ease of manufacture and scale up, reduction in drug toxicity because of their non-ionic nature of the surfactant, more physical and chemical stability as compared to niosomes, osmotically stable, used for targeted drug delivery of drugs. They are used for sustained as well as controlled drug delivery system^[20,21].

Mechanism of penetration across skin through provesicular system

The skin is the largest human organ covering an area of about 2 sq. m. in an average human adult and consists of three functional layers: epidermis, dermis and subcutaneous^[22]. It is also composed of blood vessels, nerve endings, appendages, fat and connective tissue. One major task of the skin is to protect the organism against the loss of endogenous substances and mechanical, chemical, microbial and physical influences^[23]. The outermost layer of the

skin, the epidermis, provides the protective properties. Out of the five layers of the epidermis, it is mainly the uppermost stratum corneum (SC), which is responsible for the permeation barrier properties of the skin^[24]. Basically there are three basic routes through which the permeation of drug takes place namely, via hair follicle, through sweat duct or across stratum corneum. It is 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 provesicular system should be converted to vesicles before the drug is released and permeates across the skin^[25]. Various types of vesicle-skin interactions observed during in vitro studies using human skin which may induce various effects on dermal or transdermal drug delivery^[26]. There is no such single mechanism that can sufficiently explain the mechanism involved in the permeation of drug containing vesicles into skin but many hypothesis exist that explains the permeation of vesicles through skin as shown in Figure 2:

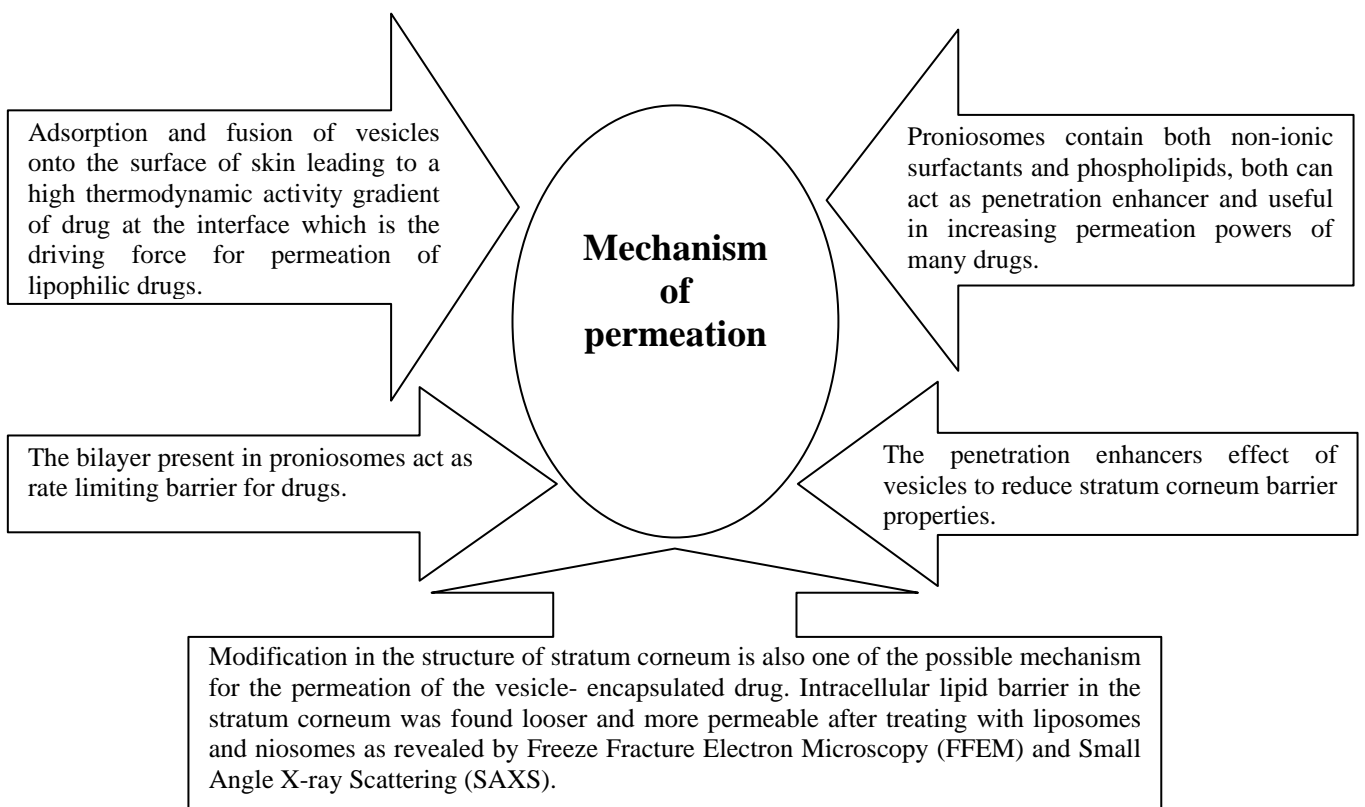


Figure 2: Mechanism of permeation through vesicles across the skin

In addition to these mechanisms the other factors which could explain the ability of vesicles to modulate drug transfer across skin, includes:^[27]

- Nature of drug
- The lipid bi-layers of vesicles 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

Formulation aspects of proniosomes as provesicular system

Proniosomes generally comprises variety of ingredients such as non ionic surfactants, phosphatidyl choline (PC), alcohol, aqueous phase, cholesterol and miscellaneous (DCP, maltodextrin, sorbitol *etc.*). The maltodextrin and sorbitol act as coating carrier^[28,29]. The ingredients along with their uses are enlisted in Table 1.

Table 1: Commonly used components for proniosomes formulation

S. No	Components	Examples	Uses
1	Surfactants	Tween (20, 40, 60, 80), span (20, 40, 60)	To increase drug flux across the skin ^[30]
2	Cholesterol	Cholesterol	To prevent leakage of drug formulation.
3	Lecithin	Soya and egg lecithin	Penetration enhancer ^[31]
4	Sugar	Maltodextrin, sorbitol	Provides flexibility in surfactant and other component ratio alters the drug distribution.
5	Solvent	Ethanol, methanol, propanolol isopropanolol,	Skin permeation
6	Aqueous phase	Hot water, buffer, glycerol	Entrapment efficiency

Non ionic surfactants

A wide range of surfactants are available and the selection 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^[32]. 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^[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].

Lecithin

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^[35]. 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].

Cholesterol

Cholesterol is an essential component of vesicles. Incorporation of cholesterol influence vesicles stability and permeability^[37]. 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]. On further increase in cholesterol beyond certain concentration level starts

disrupting the regular bi-layered structure leading to loss of drug entrapment and permeation^[39].

Solvent

Selection of solvent is another important aspect as it has great effect on vesicle size and drug permeation rate^[40]. 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 isopropanol, may be due to branched chain present in it^[41]. Ethanol may cause the reduction of lipid polar head interactions within the membrane, thereby increased the skin permeation^[42,43].

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^[44,45].

Methods of formulation of proniosomes

Proniosomal formulations can be prepared by mainly three methods such as slurry method, slow spray coating method and 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 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±2° 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 tightly closed container under refrigeration in light^[46,47].

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 flask 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 65-70°C 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^[48,49].

Coacervation phase separation method

Accurately weighed or required amount of surfactant, carrier (lecithin), cholesterol and drug can be taken in a clean and dry wide mouthed glass vial (5 ml) and solvent should be added to it. All these ingredients has to be heated and after heating all the ingredients should be mixed with glass rod. To prevent the loss of solvent, the open end of the glass vial can be covered with a lid. It has to be warmed over water bath at 60-70°C for 5 minutes until the surfactant dissolved completely. The mixture should be allowed to cool down at room temperature till the dispersion get converted to a proniosomal gel^[50,51].

The factors such as surface chain length, drug concentration, cholesterol content, charge of the lipid, total lipid concentration and pH of the medium affects the formulation of provesicles.

Characterization of proniosomes

Provesicles are characterized for vesicle size and morphology, entrapment efficiency, *In vitro* release

and permeation studies, *In vivo* studies, stability studies *etc.*

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^[52,53].

Optical microscopy

In optical microscopy, small amounts of the formed niosomes are spread on a glass slide and examined for the vesicles structure and the presence of insoluble drug crystals using ordinary light microscope with varied magnification power 100x. Photomicrographs are taken for niosomes using Nikon coolpix S220 digital camera with 6x optical zoom^[54,55].

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 vacuum evaporator and examined using a Scanning electron microscopy equipped with a digital camera at 25kV accelerating voltage^[56,57].

Transmission electron microscopy

The morphology of hydrated niosome dispersion prepared from proniosome was also determined using transmission electron microscopy (TEM). The niosome 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^[58,59].

Entrapment efficiency

Various methods can be used to evaluate the loading capacity of proniosomal systems such as dialysis method, gel filtration and centrifugation method.^[60,61] In dialysis method, amount of entrapped drug can be obtained by subtracting the amount of untrapped drug from total drug incorporated.

Entrapment efficiency (EE) = Amount of drug entrapped/total amount of drug × 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^[62,63].

***In vitro* release and permeation studies**

In vitro release and skin permeation studies for proniosomes were determined by different techniques like franz diffusion cell, dialysis tubing and reverse dialysis^[64,65]. In case of dialysis, the prewashed dialysis tubing is used which can be hermetically sealed, the proniosomes are placed in it and then dialysed against a suitable dissolution medium at a room temperature. The samples are withdrawn from the medium at suitable interval, centrifuged and analysed spectrophotometrically (UV, HPLC)^[66]. In case of Franz diffusion cell, albino

rat skin is sandwiched securely between donar and receptor compartment with the epidermis site facing the donar compartment. The receptor compartment is filled with buffer solution which is continuously stirred and thermostated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ throughout the experiment. Before starting the experiment the donar cell was sealed with parafim film and covered with aluminum foil to prevent exposure to light. At predetermined time interval for 24 hr aliquots are withdrawn and are replaced with an equal volume of fresh buffer to ensure sink condition and drug content can be determined by spectrophotometrically^[67,68].

In vivo studies

In vivo studies can be carried out by using different grades of animals *i.e.*: rats, mice, rabbits and guinea pig^[69,70].

Stability studies

Stability studies are carried out by storing the proniosomes at various temperature conditions like refrigeration, room temperature and elevated temperature according to ICH guidelines^[71]. Drug content and variation in the average vesicles diameter is periodically monitored. According to ICH guidelines the stability studies for dry proniosomes powder should be studied for accelerated stability at 75% relative humidity as per international climate zones and climate conditions^[72,73].

Applications of proniosomes as a carrier system

The application of proniosomal technology is widely varied and can be used as promising carrier for treatment of number of diseases.

Drug targeting

Proniosomes have ability to target drugs. A carrier system (such as antibodies) can be attached to niosomes (as immunoglobulin bind readily to the lipid surface of the niosome) to target them to specific organs. Many cells also possess the intrinsic

ability recognize and bind specific carbohydrate determinants, and this can be exploited by niosomes to direct carrier system to particular cells^[74].

Localized drug action

Drug delivery through niosomes 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 niosome are taken up by mononuclear cells resulting in localization of drug, increase in potency and hence decrease both in dose and toxicity^[75].

Proniosomes as a carrier

Proniosomes are used as a carrier for delivery of peptide drugs and haemoglobin within the blood. They are also used in studying immune response due to their immunological selectivity, low toxicity and greater stability. The proniosomes are also used as a carrier system in cardiac disorders, hormonal therapy, NSAIDS and antibacterial therapy^[76].

Sustained release

Sustained release action of proniosomes can be applied to drugs with low therapeutic index and low water solubility since those could be maintained in the circulation via niosomal encapsulation^[77].

Transdermal drug delivery systems utilizing proniosomes

Proniosomes 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 proniosomes. The penetration of the drugs through the skin is greatly increased as compared to un-entrapped drug. Recently, transdermal vaccines

utilizing niosomal technology is also being researched^[78].

Cosmetics or Cosmeceuticals

Proniosomes exists in two forms, *i.e.*: semisolid liquid crystal gel and dry granular powder, depending on their method of preparation. Out of these two forms, the proniosome gel is mainly used for topical/transdermal applications. Proniosome gel can be used as-an effective delivery systems for cosmetics

and Cosmeceuticals due to their unique properties^[79]. For applying therapeutic and cosmetic agents onto or through skin requires a non toxic, dermatologically acceptable carrier, which not only control the release of the agent for prolong action but also enhances the penetration to the skin layer. Proniosomes gel formulation shows advantages in controlled drug delivery improved bioavailability, reduced side effects and entrapment of both hydrophilic and hydrophobic drugs^[80].

Table 2 : Application of proniosomes as a carrier

S. No	Drug	Category	Carriers used	Results	Reference
1	Flurbiprofen	NSAIDS	Span(20,40,60,80), absolute ethanol, cholesterol	The drug release rate from cholesterol free proniosomes was found to be high.	81
2	Levonorgestrel	Anti contraceptive	Span 40, alcohol, cholesterol. Lecithin	The study demonstrated the utility of proniosomal transdermal patch bearing levonorgestrel for effective contraceptive.	82
3	Piroxicam	NSAIDS	Span 60, ethanol, cholesterol, lecithin	Span 60 based lecithin vesicle showed increased drug delivery from lipid vesicles.	83
4	Celecoxib	Cyclooxygenase inhibitor	Spans, cholesterol, lecithin, methanol, ethanol	Enhanced bioavailability of celecoxib.	84
5	Haloperidol	Neuroleptic drug	Surfactants, alcohol, cholesterol	Incorporation of single surfactant can increase drug permeation across the skin as compare to mixture of surfactants.	85
6	Ketorolac	NSAIDS	Span 60, ethanol, cholesterol, lecithin	The drug entrapment was high within the lipid bilayers of vesicles.	86
7	Captopril	Antihypertensive	Surfactants, cholesterol, lecithin, alcohol	Prolonged release of captopril.	87
8	Estradiol	Steroids	Span, tween, lecithin, cholesterol,	The non ionic surfactants in proniosomal formulations help in increasing the drug permeation across the skin.	88
9	Losartan potassium	Antihypertension	Span(20,40,60,80), tween(20,40,60), cholesterol	Enhanced bioavailability and skin permeation of drug.	89
10	Chlorpheniramine maleate	Antihistamine	Span 40, alcohol, cholesterol, lecithin	Span 40 proniosomes showed optimum stability, loading efficiency, particle size and suitable release kinetics for transdermal delivery of drug.	90
11	Tenoxicam	NSAIDS	Tween 20, spans, cholesterol, alcohol	Lecithin free proniosomal gel shows highest stability and entrapment efficiency.	91
12	Alprenolol hydrochloride	Antihypertensive	Maltodextrin, surfactants, cholesterol, alcohol	The use of the maltodextrin in proniosome formulation helps in the drug release.	92

CONCLUSION

The provesicular systems have been gaining a lot of interest of various researchers and scholars, 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. Proniosomes contain both non ionic surfactant and phospholipids, both can act as penetration enhancer and useful in increasing permeation of many drugs. However future experiments should explore the suitability of proniosomes with more drugs designed for improved and effective intended therapy. So, that proniosomes are represented as promising drug carrier and promising drug delivery module.

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