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PRONIOSOMES AND ETHOSOMES: NEW PROSPECT IN TRANSDERMAL AND DERMAL DRUG DELIVERY SYSTEM

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ABSTRACT

The present article is a descriptive study of the performances of ethosomes and proniosomes as specialized delivery systems for transdermal drug delivery system. Vesicular systems, such as ethosomes and proniosomes are used in cosmetic and pharmaceutical products to encapsulate ingredients, to protect ingredients from degradation, to increase bioavailability, and to improve cosmetic performance. A review of literature is presented here and a sincere attempt has been made to highlight the properties and characteristics of proniosomes and ethosomes in transdermal drug delivery and cosmetic/cosmeceuticals applications. Interaction studies between proniosomes and ethosomes components and skin is also discussed along with the formulation aspects of proniosomes and ethosomes formulation. Our aim is to introduce and explore proniosome and ethosomes as a carrier system for various applications of drugs and cosmeceuticals. The goal of this study is to investigate the efficiency of transcellular delivery of drugs with the help of ethosomes and proniosomes.

INTRODUCTION: Transdermal therapeutic systems are the recently developed devices, which are non invasive to skin as compared to other routes for administration of drugs. Although the skin, particularly the stratum corneum presents a barrier to most drug absorption, it provides a large (1-2 mtr^2) and accessible surface area for drug diffusion. Various types of transdermal therapeutic systems are utilized for long term continuous infusion of therapeutic agents, including antihypertensives, antifungal, analgesics, steroids and contraceptive drugs. Although transdermal delivery is currently limited to few drugs, it has achieved considerable commercial success. Some drugs which are used in transdermal delivery systems include nitroglycerine, scopolamine, estradiol, testosterone, nicotine, clonidine and estrogen-progestin combination into transdermal products [1]. Various types of transdermal drug delivery system include liposomes, erythrosomes, liposomes, niosomes, ethosomes, and proniosomes [2].

Nano- erythrosomes: an erythrocyte based new drug carrier, has been developed which is prepared by extrusion of erythrocyte ghosts to produce small vesicle having average diameter of 100µm.

Liposomes: small vesicle of a bilayer of phospholipids encapsulating an aqueous space ranging from 0.03-10µm in diameter.

Niosomes: are non-ionic surfactants based multilamellar or unilamellar vesicles in which an aqueous solution of solute(s) is enclosed by a membrane resulted from the organization of surfactant macro-molecule as bilayer.

Proniosomes and ethosomes are recent development made in transdermal therapeutic systems. These are

most advance devices which ignore demerits of liposomes and niosomes such as;

1. Liposomes require special precautions and conditions for formulation and preparations
2. Complex method for routine and large scale production
3. Less chemical stability
4. High cost and while niosomes possesses demerits like;
    1. Fusion,
    2. Aggregation,
    3. Sedimentation
    4. Leakage on storage
    5. Physical instability [3]

Proniosomes: Proniosomes are dry formulation of water-soluble carrier particles that are coated with surfactant and can be measured out as needed and dehydrated to form niosomal dispersion immediately before use on brief agitation in hot aqueous media within minutes. The resulting niosomes are very similar to conventional niosomes and more uniform in size.

Interaction Between skin and proniosomes: There is a direct contact of proniosome formulation with skin after applies, 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 non ionic 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, where as biosynthesis and turnover rates of phospholipids were increased two to four times [4].

Preparation of proniosome: Carrier which is selected for proniosomes preparation should have following characteristics like free flow ability, non-toxicity, poor solubility in the loaded mixture solution and good water solubility for ease of hydration. Different carriers and non ionic surfactants and membrane stabilizers used for the proniosome preparation are shown in **table 1** below;

TABLE 1: NON-IONIC SURFACTANTS AND COATING CARRIERS USED FOR THE PREPARATION OF PRONIOSOMES

S. No.	Non ionic surfactants used
1	Span 20
2	Span 40
3	Span 60
4	Span 80
5	Span 85
6	Tween 20
7	Tween 60
8	Tween 80
	Coating materials investigated
1	Sucrose stearate
2	Sorbitol
3	Maltodextrin
4	Maltodextrin
5	Glucose monohydrate
6	Lactose monohydrate
7	Spray dried lactose
	Membrane stabilizers used
1	Cholesterol
2	Lecithin

There are 3 methods for preparation:

1. **Slurry method**: Maltodextrin powder as carrier is added to a 250-mL round-bottom flask and the entire volume of surfactant solution was added directly to the flask to form slurry. If the surfactant solution volume is less, then additional amount of organic solvent can be added to get slurry. The flask

was attached to the rotary evaporator and vacuum was applied until the powder appeared to be dry and free flowing. The flask was removed from the evaporator and kept under vacuum overnight. Proniosome powder was stored in sealed containers at 4°C. The time required to produce proniosomes is independent of the ratio of surfactant solution to carrier material and appears to be scalable [5].

2. **Coacervation phase separation method**: weighed amounts of surfactant, lipid and drug are taken in a clean and dry wide mouthed 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 [5, 6].

3. **Slow spray-coating method**: This method involves preparation of proniosomes by spraying surfactant in organic solvent onto sorbitol powder and then evaporating the solvent. Because the sorbitol carrier is soluble in the organic solvent, it is necessary to repeat the process until the desired surfactant loading has been achieved. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to form as the carrier dissolves. The resulting niosomes are very similar to those produced by conventional methods and the size distribution is more uniform [5, 6].

Preparation of niosomes from proniosomes by hydration: Prepared proniosome powder is weighed and filled in screw cap vials. Water or saline at 80°C is added and the vials capped. The vials are attached to a vortex mixer and agitated for 2 minutes to get niosomal formulation [2].

Various methods for characterization of Proniosomes:

1. Visualization: Visualization of proniosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM)

2. Vesicle size and side distribution: Optical microscopy, laser diffraction particle size analyzer, coulter submicron size analyzer.

3. Shape & surface morphological characterization: Optical microscopy, transmission electron microscope, scanning electron microscope.

4. Angle of repose: Funnel method

5. Rate of hydration: Neubaur's chamber.

6. Drug Content : Drug can be quantified by a modified HPLC method

7. Penetration and Permeation Studies: Depth of penetration from ethosomes can be visualized by confocal laser scanning microscopy (CLSM)

Factors affecting physical nature of proniosomes: There are some factors such as hydration temperature, choice of surfactant, nature of membrane, nature of drug, etc., can affect significantly the physical nature of proniosomes (**fig. 1**).

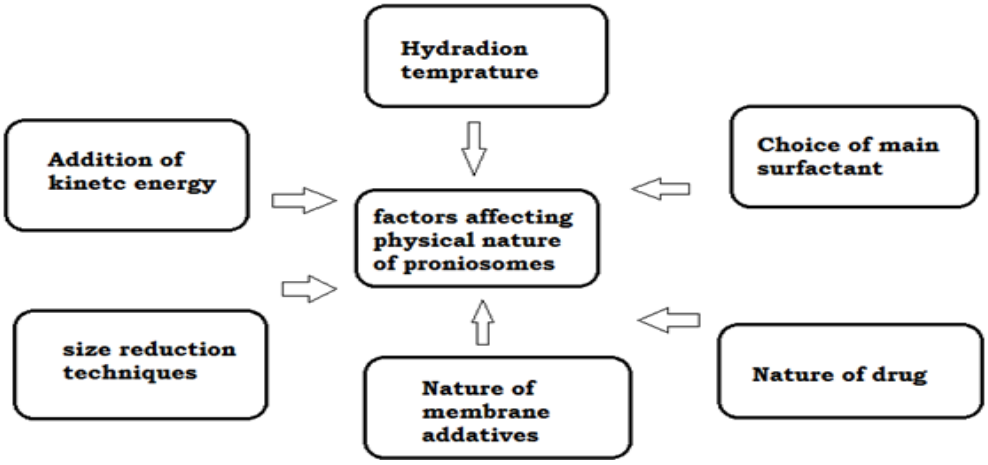

FIG. 1: FACTORS AFFECTING PHYSICAL NATURE OF PRONIOSOMEs

Ethosomes: Ethosomes are lipid-based elastic vesicular systems embodying ethanol in relatively high concentrations which enhance the topical drug delivery. The presence of ethanol prolongs the physical stability of the ethosomes with respect to liposomes. The enhanced delivery of actives incorporated in the ethosomes can be ascribed to the interactions between ethosomes and skin lipids. That may open the new pathways due to the malleability and fusion of ethosomes with skin lipids, which results in the penetration of drug into deeper skin layers.

Interaction between skin and ethosomes: The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the 'ethanol effect', whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer. This is followed by the 'ethosomes effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin [7].

Preparation of ethosomes:

Cold method: Ethosomes can be prepared from soybean phosphatidylcholine (Phospholipon 90), ethanol, drug and distilled water. Phospholipon 90 and drug should be dissolved in ethanol. Water has to be added in small quantities and the preparation mixed by mechanical stirring under controlled conditions. Ethosomal formulations were prepared according to the method reported by Touitou. Phospholipid and drug or fluorescent probe (Rhodamine-123) was dissolved in ethanol.

This mixture was heated to 30°±1°C in a water bath. Double-distilled water heated to 30°±1°C was added slowly as a fine stream to lipid mixture with constant stirring (mechanical stirrer; Remi Equipment; Mumbai, India) at 700 rpm in a closed vessel. Mixing was continued for an additional 5 minutes, while maintaining the system at 30°±1°C. The resulting vesicle suspension was homogenized by passing through polycarbonate membrane of 400, 200, or 100 nm according to initial size of formulation using hand extruder (ore, Billerica, MA) for 3 cycles [8].

Hot method: In this method, phospholipid is dispersed in water by heating in a water bath at 40^{o}C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40^{o}C. Once both mixtures reach 40^{o}C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties [69, 70]. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method [23].

Various methods for characterization of Ethosomes:

1. Visualization: Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM).

2. Vesicle size and Zeta potential: Particle size and zeta potential can be determined by dynamic light

scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS).

3. Entrapment Efficiency: The entrapment efficiency of drug by ethosomes can be measured by the ultracentrifugation technique.

4. Transition Temperature: The transition temperature of the vesicular lipid systems can be determined by using differential scanning colorimetry.

5. Surface Tension Activity Measurement: The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

6. Vesicle Stability: The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM.

7. Drug Content: Drug can be quantified by a modified high performance liquid chromato-graphic method.

8. Penetration and Permeation Studies: Depth of penetration from ethosomes can be visualized by confocal laser scanning microscopy (CLSM).

Significance of proniosomes and ethosomes over other liposomal vesicles in transdermal drug delivery system and traditional drug delivery systems: Adsorption and fusion of proniosomes or ethosomes on to the surface of skin leading to a high thermodynamic activity gradient of drug at the interface, which is the driving force for permeation of lipophilic drugs [6] (**fig. 2**). The effects of ethosomes and proniosomes vesicles as the permeation enhancer reduce he barrier properties of stratum corneum. The lipid bilayers of niosomes act as rate limiting membrane barrier for drugs, stratum corneum in transdermal delivery.

These were non thermoresponsive at 30°C and extremely viscous, hence if either the ambient temperature or the skin temperature were raised to 35°C, they were capable to release their encapsulated contents.

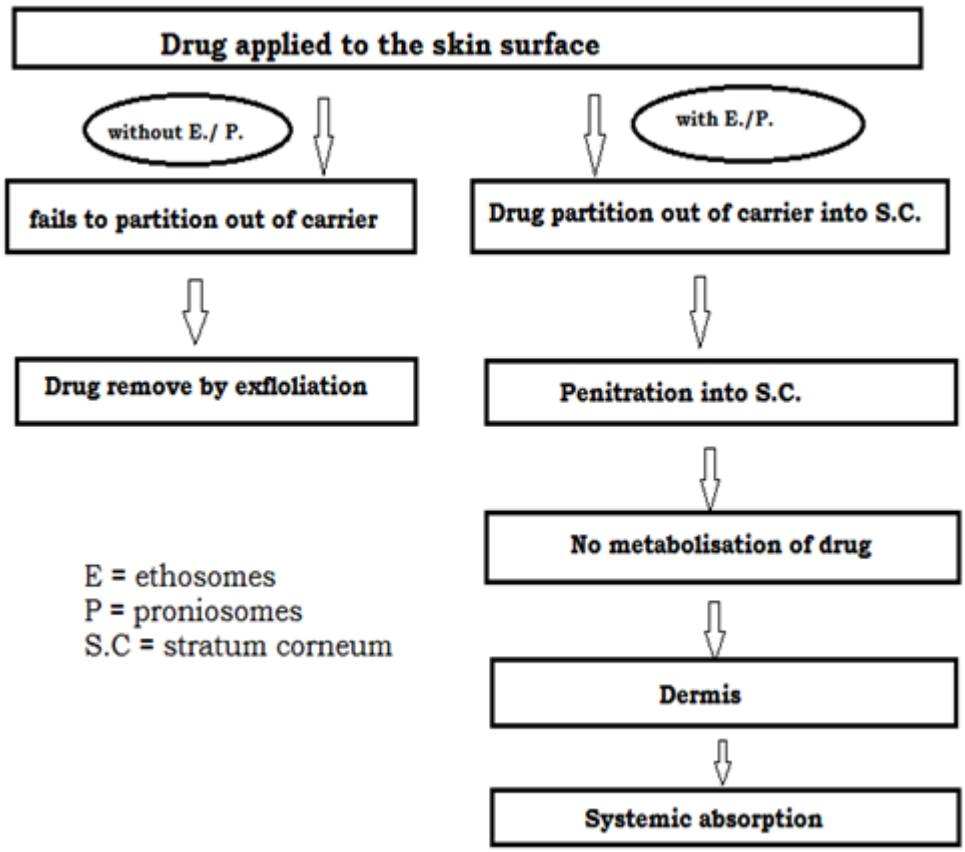

FIG. 2: MECHANISM OF ACTION OF PRONIOSOMES AND ETHOSOMES

Significance of proniosomes: Significance of proniosomes can be describes on the basis of different studies related to specific applications of proniosomes as a carrier system in transdermal delivery of different drugs which are as follows;

- The polynomial and contour plots developed by using central composite design allowed to prepare proniosomes with optimum characteristic of Aceclofenac, a lipophilic NSAID.
- The formulation by single surfactant with proniosomes increased the permeation of Haloperidol, a hydrophilic Antipsychotic drug.
- There was an increased drug delivery from proniosome vesicle than span 60 based lecithin vesicle for piroxicam, a lipophilic NSAID.
- Proniosomes capable to efficiently deliver entrapped drug delivery an extended period of time.
- Proniosomes derived niosomes are superior in their ability to release the Ibuprofen, a lipophilic NSAID at a constant rate [9].
- The use of maltodextrin in proniosomes helps in enhancement of drug release of Alprenolol Hydrochloride, a lipophilic Antihypertensive [10].
- The release rate of drug with proniosomes vesicle of Indomethacin, a lipophilic NSAID was studied in controlled manner [11].
- Prolonged release of captropril with proniosomes was studied significantly [12].
- Enhanced absorption of Griseofulvin, a lipophilic Antifungal with proniosome vesicle [13].
- In Flurbiprofen, a lipophilic NSAID, the drug release ratio from cholesterol free proniosome was found to be high [14].
- The non-ionic surfactant in proniosomal formulation helps in enhancement of drug

permeation through the skin in the case of Estradiol, a lipophilic drug which is used in usual symptoms of menopause [15].

- In Ketorolac, a lipophilic NSAID, the drug entrapment was high within the lipid bilayer of proniosome vesicles [16].
- Proniosome enhanced bioavailability & skin permeation of Losartan Potassium, a hydrophilic antihypertensive drug [17].
- The study demonstrated the utility of proniosomal transdermal patch bearing Levonorgestrol for effective contraception [18].
- Proniosome adds enhanced bioavailability of Celecoxib, a lipophilic COX inhibitor [19].
- High nebulisation efficiency percentage and good physical stability were observed with proniosome vesicle of Cromolyn Sod., a hydrophilic Antiasthmatic and Antiallergic drug [20].
- Span 40 proniosomes showed optimum stability, loading efficiency and particle size and release kinetics suitable for transdermal delivery of Chlorpheniramine maleate, a hydrophilic Antihistaminic drug [21].
- Proniosomes gel can be used as an effective delivery system for Cosmetics due to their unique properties.

On the basis of above description following advantages of proniosomes can be illustrated In comparison to other transdermal & dermal delivery systems:

1. The proniosome minimizes these problems by using dry, free-flowing product, which is more stable during sterilization and storage.
2. Easy transfer, distribution, measuring, and storage make proniosomes a versatile delivery system with potential for use with a wide range of active compounds.
3. The great advantage offered by proniosomes is their ease of use and their hydration is much easier than the long shaking process required to hydrate surfactants in the conventional dry film method.
4. Furthermore, unacceptable solvents are avoided in proniosomal formulations. The systems may be directly formulated into transdermal patches and doesn't require the dispersion of vesicles into polymeric matrix.

Significance of ethosomes: Significance of ethosomes can be describes on the basis of different studies related to specific applications of ethosomes as a carrier system in transdermal delivery of different drugs which are as follows;

- Hair follicles and sebaceous glands are increasingly being recognized as potentially significant element in percutaneous drug delivery. Ethosomes are used by Meiden *et al*., for targeting these [2, 22].
- Oral administration of hormone is associated with problems like first pass metabolism, low oral bioavailability and several dose dependent side effects. Touitou *et al*., observed nearly 30 times higher skin permeation of testosterone from ethosomal formulation [11].
- Better skin permeation potential of ethosomal Trihexyphenidyl hydrochloride formulation, an Antiparkinson formulation [23].
- Ethosomes as an attractive clinical alternative for Anti HIV therapy because of better cellular uptake [8].
- Topical delivery of Anti-arthritis drug is a better option for its site specific delivery and overcomes the problem associated with conventional therapy [22].
- Encapsulation of Cannabilol in ethosomes increase its skin permeation, accumulation and hence its biological activity [11].
- Ethosome- Insulin formulation provides control release [22].

- Ethosome formulation of Antibiotics like erythromycin shows improved skin deposition and biological activity with prolonged drug action [23].
- Anti HIV agents like Zidovudine, Lamivudine shows improved transdermal flux, biological activity & reduced drug toxicity [22].
- Ethosomal-Azelaic acid formulation shows prolonged release [23].
- Ethosomal Ammonium glycyrrhininate shows improved dermal disposition, exhibiting sustained release improved [24].
- Ethosomal Minodixil formulation shows higher skin retention [11].
- Ethosomes has proven to be superior for topical administration of Aceclofenac.
- Ethosomes transdermal patches reported remarkably enhances bioavailability and stability of peptide drugs than the oral formulation [11].
- The ethosomal Ciclopirox olamine transdermal formulation is prepared and showed that it enhances the dermal penetration.

On the basis of above description following advantages of ethosomes can be illustrated In comparison to other transdermal & dermal delivery systems:

1. Ethosomes are enhanced permeation of drug through skin for transdermal and dermal delivery.
2. Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules)
3. Ethosome composition is safe and the components are approved for pharmaceutical and cosmetic use.
4. Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.
5. High patient compliance- The Ethosomal drug is administrated in semisolid form (gel or cream), producing high patient compliance by is high. In contrast, Iontophoresis and Phonophoresis are relatively complicated to use which will affect patient compliance.
6. High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes.
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
8. Various application in Pharmaceutical, Veterinary, Cosmetic field.

CONCLUSION: Ethosomes have been found to be much more efficient at delivering drug to the skin, than either liposomes or hydroalcoholic solution. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. While Proniosomes contain both non-ionic surfactant and phospholipids, both can act as penetration enhancer and useful in increasing permeation powers of many drugs.

A wide variety of active agents of different therapeutic functions were formulated into proniosomal and ethosomes in transdermal and dermal drug delivery system. So on the basis of these studies we concluded that ethosomes and proniosomes are the present and future of vesicle system in transdermal and dermal delivery of various drugs.

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