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A REVIEW ON ETHOSOMES AS A NOVEL DRUG DELIVERY SYSTEM

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Abstract: As the ethosomal systems are conceptually sophisticated, they are characterized by simplicity in their preparation, safety & efficacy a combination that can expand highly their application. The ethosomes are soft, malleable vesicles tailored for the enhanced delivery of the active agents. The ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers or/and the systemic circulation. The ethosomes are gaining popularity in designing the drug delivery systems for the topical & transdermal use for their capability to reach the deep skin layers & systemic circulation. This review article focuses on various aspects of ethosomes like, their method of preparation, mechanism of penetration, characterization, advantages, composition, marketed products & applications of ethosomes.

Keywords: Ethosomes, Transdermal, Mechanism of Penetration

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INTRODUCTION

Due to its noninvasive procedure for the administration, the transdermal drug delivery is gaining importance. The transdermal drug delivery overcomes the number of limitations of the oral drug delivery such as the irritation of gastrointestinal mucosa, degradation of drugs by digestive enzymes & the first pass effect. The Patients also highly prefer transdermal route due the pain on administration associated with the parenteral route. The transdermal dosage forms enjoy being the most patient compliant mode of delivery of the drug. [1, 2]

CHALLENGES WHILE DESIGNING THE TRANSDERMAL DOSAGE FORMS

The skin is the multi-layered structure made up of the stratum corneum, the outermost layer, under which lies the epidermis & dermis. Within these layers of the skin are interspersed fibroblasts, hair follicles & the sweat glands that originate in the blood supply of dermis. The almost unsurmountable nature of the stratum corneum is the major challenge for the systemic delivery of the percutaneously applied drugs. The Obrick & the mortaro arrangement of the corneocytes, the flattened mononucleated keratinocytes, with the interspersed lipids & the proteins makes the stratum corneum approximately 1000 times less permeable than the other biological membranes. It is even more difficult for anything to penetrate to the deeper strata of the skin. [3-5]

NEED FOR TRANSDERMAL DRUG DELIVERY

Transdermal Drug delivery offers the several unique advantages including relatively large & readily accessible surface area for the absorption, the ease of application & termination of the therapy, despite the different challenges. For delivering of the drug molecules, the evolution of the better technologies, the use of vesicular carriers, safe penetration enhancers have rejuvenated the interest for designing the transdermal drug delivery system for the drugs that were thought to be unfit for the transdermal delivery of drug.

ETHOSOMES

Ethosomes are the vesicular carrier consisting of the hydroalcoholic or the hydro / alcoholic/ glycolic phospholipids in which the alcohols concentration or their combination is high relatively. With various chemical structures like the hydrogenated PC, phosphatidylcholine [PC], phosphatidylserine [PS], phosphatidic acid [PA], phosphatidylinositol [PI], phosphatidylethanolamine [PE], alcohol [ethanol or isopropyl alcohol], phosphatidylglycerol [PPG], the water & the propylene glycol [or other glycols] the ethosomes may contain phospholipids. A composition like this enables the delivery of the high concentration of the active ingredients through the skin. The change in the alcohol: water or alcoholpolyol: water ratio alters the delivery of drug. The soya phospholipids such as PL-90 [Phospholipon 90] in

concentration range of 0.5-10% w/w, is the phospholipids generally used. The cholesterol at the concentrations ranging between the 0.1-1% can also be used in the preparation to increase the stability of the ethosomes. Alcohols like isopropyl alcohol & ethanol & the glycols like the Transcutol & the propylene glycol are generally used. Sometimes the non-ionic surfactants [PEG-alkyl ethers] in the combination with the phospholipids are used in these preparations. Cationic lipids like dodecylamine, cetrimide, POE alkyl amines, cocoamide, etc. can also be included. In the final product the concentration of the alcohol may range from 20 - 50%. The non-aqueous phase [alcohol & glycol combination] concentration may range between 22- 70%. [6]

COMPOSITION OF ETHOSOMES

The ethosomes are mainly composed of the high concentration of hydroalcohols or hydroalcohols, phosphatidylcholine, glycols & water. The Phosphatidylcholine can be, phosphatidyl soya phosphatidylcholine, dipalmityl phosphatidyl choline, egg phosphatidylcholine, hydrogenated phosphatidylcholine. As the alcohols, we can use ethanol or the isopropyl alcohol & as poliglicols propylene glycol & transcutol [7]

Table 1: Different Additives Employed In Formulation of Ethosomes. [6]

Class	Uses	Example
Phospholipid	Vesicles forming component	Soya phosphatidyl choline
		Dipalmitylphosphatidyl choline
		Egg phosphatidyl choline
		Distearylphosphatidyl choline
Alcohol	For providing the softness for vesicle membrane	Ethanol
	As a penetration enhancer	Isopropyl alcohol
Dye	For characterization study	Rhodamine-123
		Rhodamine red
		FluoresceneIsothiocynate (FITC)
		6- Carboxy fluorescence
Polyglycol	As a skin Penetration enhancer	Propylene glycol
		Transcutol RTM
Cholesterol	For providing the	Cholesterol

	stability to vesicle membrane	
Vehicle	As a gel former	Carbopol D934

ETHANOL- AS PENETRATION ENHANCER

The substances that reduce reversibly the barrier resistance of the stratum corneum are known as the chemical penetration enhancers. One of the most commonly used permeation enhancers is the ethanol. Several numbers of mechanisms have been proposed for the permeation enhancing action of the ethanol. To enhance the solubility of the drug the ethanol can be included in the formulation as the solvent. For poorly soluble permeants this is particularly important as they are prone to depletion in the donor vehicle. The ethanol is the relatively volatile solvent & will rapidly evaporate at the skin temperature. The loss of ethanol from the formulation may lead to the drug becoming supersaturated, which will influence drug flux across the membrane. In addition the ethanol is thought to alter the solubility properties of the stratum corneum facilitating improved drug partitioning. The ethanol has been employed in vitro to enhance the transdermal delivery of the levonorgesterol, hydrocortisone & the 5-fluorouracil across rodent skin & estradiol across the human skin in vivo. the enhancement effect of ethanol was concentration dependent was noted by the Megrab & the collaborators. The effect of ethanol on skin water content was investigated by the authors & concluded that the formulations containing high levels of the alcohol were capable of the dehydrating the skin which may explain the concentration dependant action of the ethanol.[8,9]

METHOD OF PREPARTION

There are two methods which can be used for the formulation & preparation of the ethosomes. These two methods are very convenient & simple & do not involve any sophisticated instrument or the complicated process. The formulation of ethosomes is done by following two methods

THE HOT METHOD

The phospholipid is dispersed in water by heating in the water bath at 40 ^{o}C until the colloidal solution is obtained in this method. Mix properly ethanol & propylene glycol & heat upto 40^{0}C in the separate vessel. Then add the organic phase into the aqueous phase. Depending on its solubility, dissolve the drug in the ethanol or water. To the desire extent by using the probe sonication or the extrusion method. the vesicle size of the ethosomal formulation can be decreased.

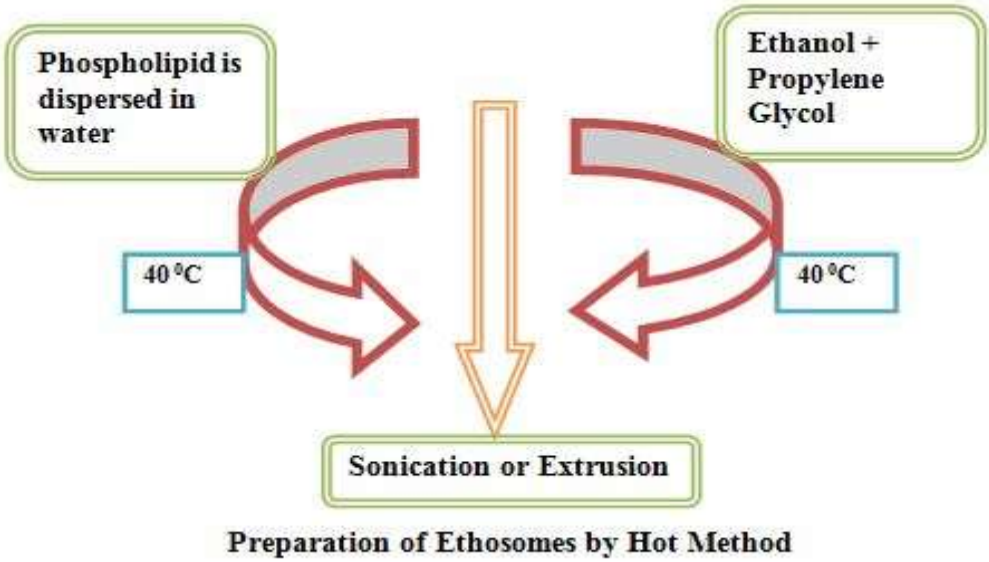

Figure 1: Preparation of Ethosomes by Hot Method

THE COLD METHOD

It is the most widely & commonly used method for the preparation of ethosomes. At room temperature dissolve the phospholipid, drug & other lipid materials in the ethanol in the covered vessel with vigorous stirring. Then during stirring add propylene glycol or the other polyol. Then in the water bath heat the mixture upto 30 ^{o}C. Then heat the water upto 30^{0}C in the separate vessel & then add to the mixture & then stir it for 5 min in the covered vessel. By using sonication or extrusion method the vesicle size of the ethosomal formulation can be decreased to the desire extend. Lastly, the formulation should be properly stored under the refrigeration. [1,4]

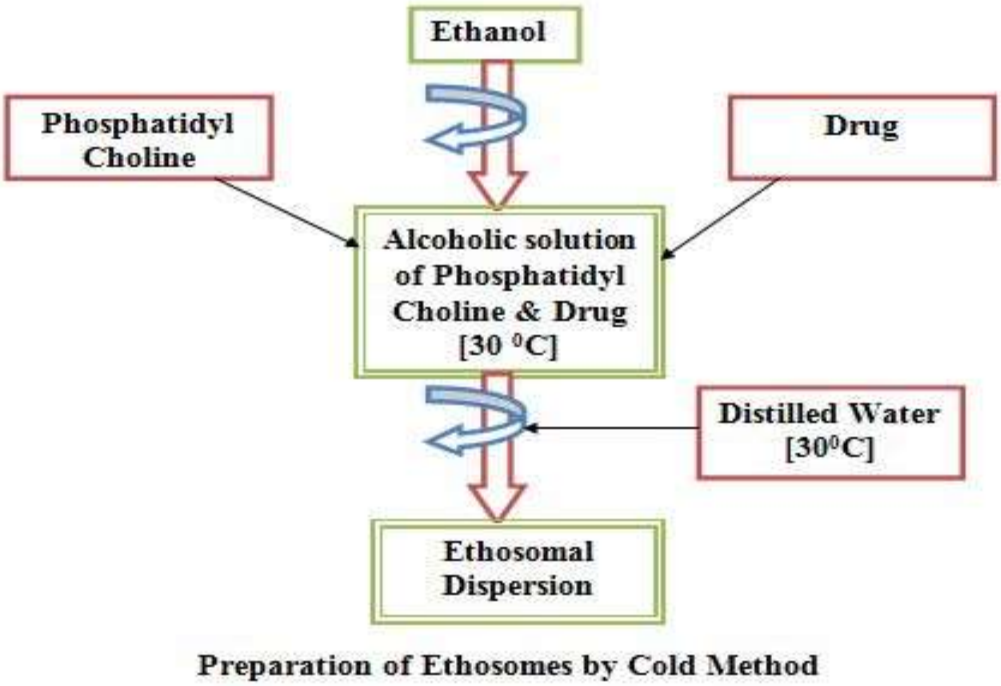

Figure 2: Preparation of Ethosomes by Cold Method

MECHANISM OF DRUG PENETRATION

The first part of the mechanism is due to the 'ethanol effect' whereby the intercalation of the ethanol into a intercellular lipids increasing lipid fluidity & decreases the density of the lipid multilayer. This is followed by the "ethosome effect" which includes permeation & the inter lipid penetration by the opening of new pathways due to the malleability & fusion of the ethosomes with the skin lipids. This Absorption of ethosomes is still not clear. in following two phases the drug absorption probably occurs,

1. The Ethanol effect

2. The Ethosomes effect

THE ETHANOL EFFECT

The ethanol is the major ingredient & acts as the penetration enhancer during the skin. The mechanism of its penetration enhancing effect is also well known. The ethanol interacts with the lipid molecules in the polar hard group region resulting in the reducing the rigidity of the stratum corneum lipids, increasing their fluidity. The intercalation of the ethanol into the polar head group environment can result in the increase in the membrane permeability. In addition, to the effect of the ethanol on the stratum corneum structure, with the stratum corneum barrier, the ethosome itself may interact.

THE ETHOSOMES EFFECT

The Increased cell membrane lipid fluidity caused by the ethanol of the ethosomes results in increased skin permeability. In the case of the drugs encapsulating ethosomes, the higher positive zeta potential imparted by the drug can improve skin attachment of the vesicles. While the encapsulated drug in the classic liposomes remained primarily at the surface of the skin. The ethosomal system was showed to be the highly efficient carrier for the enhanced drug delivery through the skin due to the increased fluidity of the lipids. [10]

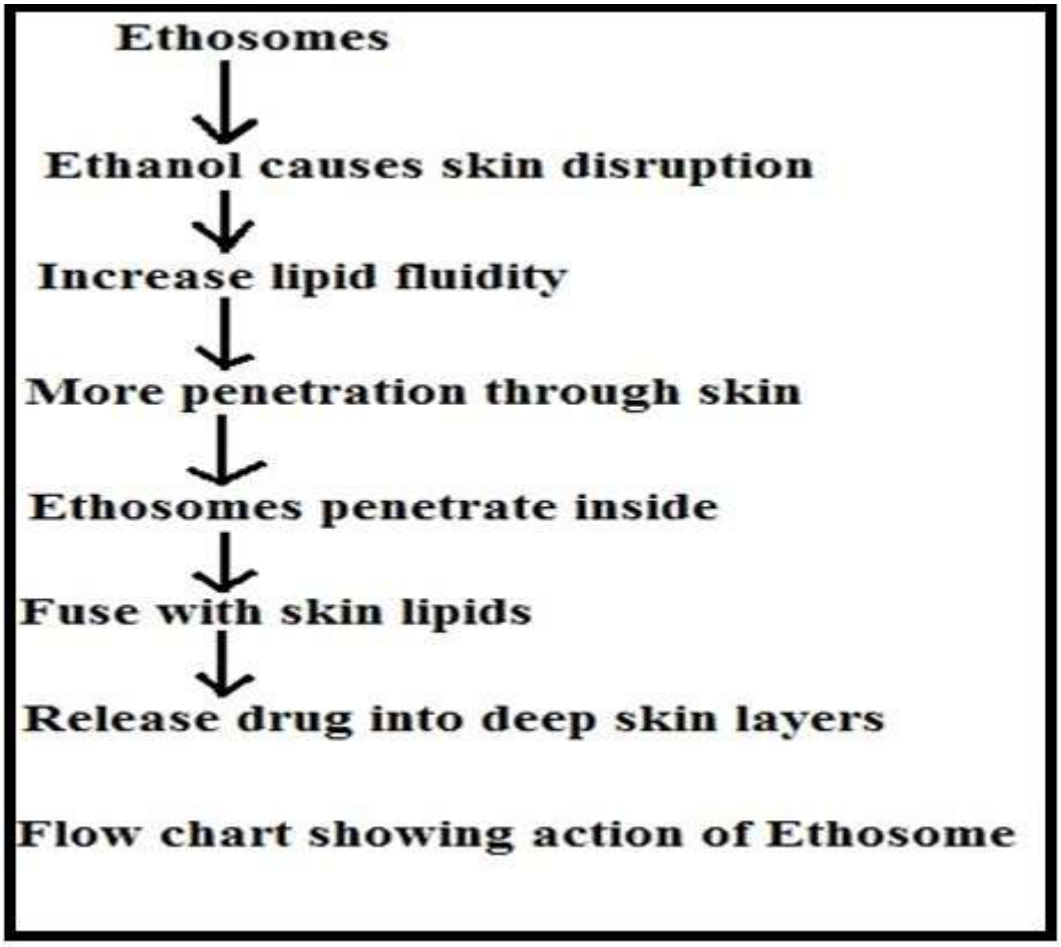

Figure 3: Flow chart showing action of Ethosomes

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY

In comparison to other transdermal & dermal delivery systems

1] The ethosomal drug delivery system can be applied widely in Veterinary, Pharmaceutical & the Cosmetic fields.

2] Patient compliance is High-The ethosomal drug is administrated in the semisolid form [cream or gel] hence it produces high patient compliance.

3] It is simple method for the drug delivery in comparison to the iontophoresis & the phosphophoresis & the other complicated methods.

4] The delivery of the large molecules [protein molecules, peptides] is possible.

5] This Ethosomal system is non-invasive, passive & is available for immediate commercialization.

6] The enhanced permeation of the drug through the skin for transdermal drug delivery.

7] It contains non-toxic raw material in the formulation. [11-13]

Table 3: Methods for the Characterization of Ethosomal Formulation

Methods	Parameters	References
Confocal laser scanning microscopy Fluorescence microscopy Transmission electron microscopy Eosin-Hematoxylin staining	Vesicle Skin interaction study	[14,15]
Extrusion method	Degree of deformability	[16, 17]
Franz diffusion cell	Drug deposition study	[16,17]
Mini column centrifugation method Fluorescence spectrophotometry	Entrapment efficiency	[18]
Dynamic light scattering method	Stability study	[10]
Transmission electron microscopy Scanning electron microscopy	Vesicle shape (morphology)	[19,20]
Nephalometer	Turbidity	[21]
Zeta meter	Zeta potential	[21]
Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion	Invitro drug release study	[19,21]
Transmission electron microscopy (TEM)	Vesicle size and size distribution	[21,22]

Scanning electron microscopy (SEM)		
31P NMR Differential scanning calorimeter	Phospholipid-ethanol interaction	[10,23]

APPLICATIONS OF ETHOSOMES

1. The Delivery of Anti-Viral Drugs

A potent antiviral agent zidovudine is acting on the acquired immunodeficiency virus. The oral administration of the zidovudine is associated with the strong side effects. Hence, an adequate zero order delivery of the zidovudine is desired to maintain the expected anti-AIDS effect [24]. The Jain *et al.* [25] concluded that ethosomes could the increase the transdermal flux, prolong the release & present an attractive route for the sustained delivery of the zidovudine. Another anti-viral drug acyclovir is topically used widely for the treatment of the Herpes labialis [26]. With poor skin penetration of the hydrophilic acyclovir to dermal layer resulting in weak therapeutic efficiency the conventional marketed acyclovir external formulation is associated. The replication of virus takes place at the basal dermis has been reported. [27]. Horwitz *et al.* formulated the acyclovir ethosomal formulation for dermal delivery, to overcome the problem associated with the conventional topical preparation of the acyclovir The results showed that higher percentage of abortive lesions & shorter healing time were observed when into ethosomes the acyclovir was loaded.

2. The Topical Delivery of DNA

Several environmental pathogens attempt to enter the body through the skin. The skin therefore has evolved into an excellent protective barrier which is also active immunologically & able to express the gene [28]. Another important application of ethosomes is to use them for topical delivery of DNA molecules to express genes in skin cells on the basis of the above facts. The Touitou et al. in their study encapsulated the GFP-CMV-driven transfecting construct into the formulation of ethosome. They applied this formulation to the dorsal skin of the 5-week male CD-1 nude mice for the 48 hour. After the 48 hr the treated skin was removed & the penetration of the GFP [Green Fluorescent Protein] formulation was observed by the CLSM. Topically applied ethosomes-GFP-CMV-driven transfecting construct enabled efficient delivery & the expression of the genes in skin cells, was observed. It was suggested that the ethosomes could be used as the carriers for the gene therapy applications that require the transient expression of the genes. These results also showed that the possibility of using the ethosomes for the effective transdermal immunization. The Gupta et al. recently reported immunization

potential using the transfersomal formulation. Hence, the better skin permeation ability of the ethosomes opens the possibility of using these dosage forms for delivery of the immunizing agents [29].

3. The Transdermal Delivery of Hormones

The oral administration of the hormones is associated with the problems like the high first pass metabolism, low oral bioavailability & several dose dependent side effects. With each pill missed the risk of failure of treatment is known to increase [30]. The Touitou *et al.* compared the skin permeation potential of the testosterone ethosomes [Testosome] across the rabbit pinna skin with the marketed transdermal patch of the testosterone [Testoderm patch, Alza]. They observed nearly 30-times higher the skin permeation of the testosterone from the ethosomal formulation as compared to that of the marketed formulation.

4. The Delivery of anti-parkinsonism agent

The Dayan & Touitou prepared the ethosomal formulation of the psychoactive drug THP [trihexyphenidyl hydrochloride] & compared its delivery with that from the classical liposomal formulation. THP is the M1 muscarinic receptors antagonist & is used in the treatment of the parkinson disease. The results indicated that the better skin permeation potential of the ethosomal-THP formulation & its use for better management of the parkinson disease [29].

5. The Transcellular Delivery

The Touitou *et al.* in their study demonstrated the better intracellular uptake of the DNA, bacitracin, & erythromycin using the CLSM & FACS techniques in different cell lines. The better cellular uptake of the anti-HIV drug zidovudine & lamivudine in MT-2 cell line from the ethosomes as compared to the marketed formulations suggested ethosomes to be an attractive clinical alternative for the anti-HIV therapy [7, 31].

6. The Delivery of Anti-Arthritis Drug

The delivery of anti-arthritis drug by topical route is the better option for its site-specific delivery & overcomes the problem associated with the conventional oral therapy. For treating the rheumatoid arthritis, the CBD [Cannabidol] is the recently developed drug candidate. The Lodzki *et al.* prepared cannabidol ethosomal formulation for the transdermal delivery. When tested by the carrageenan induced rat paw edema model, the results shows significantly increase in the biological antiinflammatory activity of the CBD-ethosomal formulation was observed. It was concluded that encapsulation of cannabidol in ethosomes significantly increased its accumulation, skin permeation & hence it's biological activity [29].

7. The Delivery of Problematic drug molecules

It is difficult for the oral delivery of the large biogenic molecules such as the proteins or peptides because in the GI tract they are completely degraded. For overcoming the problems associated with the oral delivery the non-invasive delivery of the proteins is the better option [25]. The effect of ethosomal insulin delivery in lowering blood glucose levels (BGL) *in vivo* in normal and diabetic SDI rats is investigated by the Dkeidek & Touitou. In this study the Hill Top patch containing insulin ethosomes was applied on the abdominal area of an overnight fated rat. The result shows that the insulin delivered from this patch produced the significant decrease [up to 60%] in the BGL in both normal & diabetic rats. The insulin application from the control formulation was not able to reduce the BGL On the other hand. For the treatment of inflammatory skin disease like psoriasis, atopic dermatitis and disease of hair follicle like alopecia areata etc Verma and Fahr [8] reported the cyclosporin A ethosomal formulation. The potential application of ethosomes for dermal delivery of ammonium glycyrrhizinate is investigated by Paolino *et al.* [33] The Ammonium glycyrrhizinate is naturally occurring triterpenes obtained from Glycyrrhizinate Glabra & useful for the treatment of the various inflammatory based skin diseases [34].

8. The Delivery of Antibiotics

For increasing the therapeutic efficacy of these agents the topical delivery of antibiotics is the better choice. The conventional oral therapy causes several allergic reactions along with the several side effects. The conventional external preparations possess the low permeability to the deep skin layers & subdermal tissues. By delivering sufficient quantity of antibiotic into deeper layers of skin the ethosomes can circumvent this problem. The ethosomes penetrate rapidly through the epidermis & bring appreciable amount of drugs into the deeper layer of the skin & suppress the infection at their root. With this purpose in mind the Godin & Touitou prepared the bacitracin & erythromycin loaded ethosomal formulation for the dermal & intracellular delivery. The results of this study showed that the ethosomal formulation of the antibiotic could be highly efficient & the problems associated with conventional therapy would overcome. [28]

PATENTED AND MARKETED FORMULATION OF ETHOSOME

The ethosome was invented & patented by the Prof. Elka Touitou along with her students of the department of Pharmaceutics at the Hebrew University School of Pharmacy. The NTT [Novel

Therapeutic Technologies] Inc of the Hebrew University have been succeeded in bringing the number of products to the market based on ethosome delivery system. The Noicellex TM an

anti-cellulite formulation of the ethosome is currently marketed in Japan. Another formulation, Lipoduction TM is currently used in the treatment of cellulite containing pure grape seed extracts [antioxidant] is marketed in USA. Similarly the Physonics is marketing anti-cellulite gel Skin Genuity in London. Nanominox© containing monoxidil is used as hair tonic to promote hair the growth is marketed by the Sinere. [35, 36]

CONCLUSION

The main limitation of transdermal drug delivery system i.e. the epidermal barrier can be overcomed by the ethosomes to significant extent. The ethosomes are soft, malleable vesicles & possible carrier for the transportation of the drugs. The ethosomes are characterized by the simplicity in their safety, efficacy & preparation & can be tailored for the enhanced skin permeation of the active drugs. It can be concluded that ethosomes can provide better skin permeation than the liposomes. The ethosomes have been tested to encapsulate the cationic drugs, hydrophilic drugs, proteins & the peptides. For the development of the novel improved therapies the ethosomal carrier has opened new opportunities.

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