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Ethosomes: A Potential Transdermal Drug Delivery System-In-Depth Review

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ABSTRACT

Transdermal Drug Delivery System (TDDS) has been a major area of research these days offering many advantages as compared to traditional drug delivery systems. However, TDDS has limited market success due to its barrier properties lowering success of systemic drug delivery from liposomal formulation after topical application owing to the inability of such vesicles to pass through the narrow (< 30 nm) intercellular passage in the outer skin layers. The Introduction of ethosomal technology has initiated a potentially promising area on vesicular research for effective transdermal drug delivery as preparation of ethosomes is easy with no complicated equipment involved and therefore can be scaled up to the commercial level. Ethosomes are modified lipid carriers that enable drugs to reach into deeper skin layers. They are non-invasive soft, malleable vesicles representing a lipid vesicular carrier system embodying ethanol in relatively high concentration. Compared to classic liposomes, known to mainly deliver drugs to outer layers of skin, ethosomes sufficiently penetrate through the stratum corneum delivering drugs to and across the deeper layers of skin. Many reports show a promising future of ethosomes in making transdermal delivery of various highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil, as well as cationic drugs such as propranolol, trihexyphenidyl, Cyclosporine A, insulin, salbutamol etc. Improving drug's efficacy, patient compliance, and reduction in total cost of treatment. This review includes the introduction to ethosomes, composition, and methods of preparation, characterization and also highlights recent ethosomal studies conducted in the past years 5years (2013-2017).

INTRODUCTION:

The skin is the largest organ of the human body and functions as a barrier, protecting the internal structures of the body from the environment. Structurally, skin primarily consists of the epidermis, dermis and the underlying subcutaneous fat. The epidermis forms the outermost layer, being exposed to the environment on one side and separated from the dermal tissue by a basement membrane on the other. From this basal membrane to the surface of the skin, the epidermis can be differentiated into several strata, namely: stratum germinativum or stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum[1,2].The skin acts as a barrier to all exogenous materials and restricts the entry of most of the drugs into the body. Most of the barrier function of the skin is achieved by the stratum corneum[3-5]. This presents a highly organized, brick and- mortar type of structure where keratinocytes are embedded in a lipid matrix. Permeation of drugs through this lipid matrix (the intercellular route of permeation) is considered to be a major pathway for molecules to cross the stratum corneum[6]. Due to its lipid content, the permeability of the stratum corneum to hydrophilic molecules is limited, so highly hydrophilic molecules, are unable to partition into the lipoidal stratum corneum layers and hence cannot passively permeate through skin [1].

In general, moderately lipophilic, low molecular weight compounds <500Kd are ideal candidates for transdermal permeation. To overcome the stratum corneum barrier, various studies have reported on a number of mechanisms including the use of chemical or physical and enhancers, such as sonophoresis, iontophoresis, permeation enhancers, surfactants and lipid-based systems or encapsulation technologies like Liposomes, niosomes, transferosomes and ethosomes[7-12].

Lipid-based systems offer excellent candidature for transdermal delivery due to their biocompatibility and ease of mixing with the skin lipids. There has been considerable interest in the use of liposomes for transdermal drug delivery [13,14,3]. Nevertheless, liposomes do not offer much value as they cannot penetrate into deeper layers of skin, due to their confined to the upper layer stratumcorneum[15,16]. Continuous research with the lipid-based system has resulted in the introduction of two novel carriers, transfer some sande thosomes. Transfer some sarede formable lipid vesicles consisting of phospholipid sandanedge activator which is of tenasingle chain surfactant molecule [17,6]. Ethosomes can enhance permeation through the stratum corneum barrier [18,19]. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux compared to conventional liposomes [20-22].

ETHOSOMES

Ethosomes were discovered and developed by Touitou et al.,1997[23].“Ethosomesare ethanolic liposomes”. They can be defined as noninvasive novel delivery carriers that enable transfer of drugs into and across deep skin layers and/or the systemic circulation 24.Additionally, ethosomes are vesicular carriers comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high[16].These are soft, malleable vesicles tailored for enhanced delivery of active agents. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from the immune response or other removal systems and thus be able to release just the right amount of drug and keep that concentration constant for long periods of time [24].They are a slight modification of well-established drug carrier liposome. Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water. The size range of ethosomes may vary from tens of nanometers (nm) to microns (μ)[25].

Advantages of ethosomal formulations [26,27]

1. Contains non-toxic raw material in a formulation.

2. Enhanced permeation of drug through the skin for transdermal drug delivery.

3. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.

4. High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.

5. The simple method for drug delivery in comparison toIontophoresis and Phonophoresis and other complicated methods.

6. The Ethosomal technology is available for immediate commercialization[28].

7. The main advantage of ethosomes over liposomes is the increased permeation of the drug[29-34]

Disadvantages of Ethosomal formulation[35]

They required High blood levels cannot be administered –limited only to potent molecules, those requiring a daily dose of 10mg or less.

1. Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it usually designed to offer slow, sustained drug delivery.

2. The adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.

3. The molecular size of the drug should be reasonable that it should be absorbed percutaneously.

4. An adhesive may not adhere well to all types of skin.

5. May not be economical.

6. Poor yield.

7. Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.

8. Loss of product during transfer from organic to water media.

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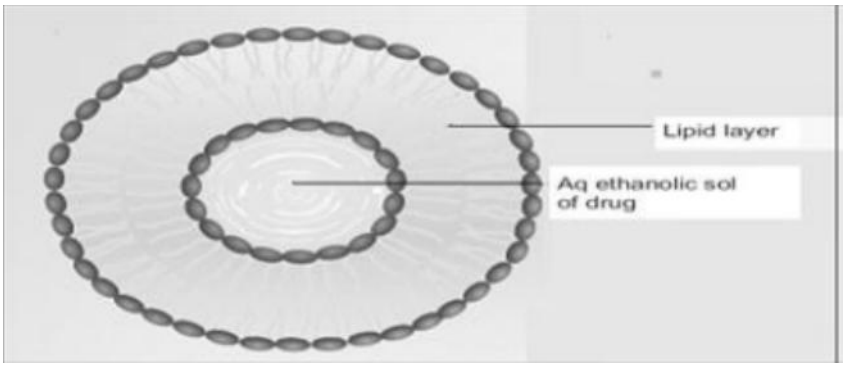

Figure 1: Ethosome structure[25]

Composition

According to Subheet et al. and many others authors have reported that ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS),

phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), hydrogenated PC,alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols) [16].Examples of alcohols applicable include ethanol and isopropyl alcohol. Among glycols, propylene glycol and Transcutol are mostly used. This composition enables delivery of high concentration of active ingredients through the skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio. Commonly preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). Mostly employed in a range of 0.5-10% w/w. Cholesterol at the concentration range of 0.1-1% can also be added to the preparation. Additionally, non-ionic surfactants (PEG-alkyl ethers) can be combined with the phospholipids in these preparations. Cationic lipids like cocamide, POE alkyl amines, dodecyl amine, can be added too. The alcohol concentration in the final product may range from 20 to 50%.The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70% as in (Table 1)below.

Table 1: Various Additives Used In Formulation of Ethosomes[36-40]

Class	**Uses**	**Example**
Phospholipid	Soya phosphatidylcholine Egg phosphatidylcholine Dipalmitoylphosphatidylcholine Distearylphosphatidylcholine	Vesicles forming component
Vehicle	gel former	CarbopolĐ934
Polyglycol	As a skin penetration enhancer	Propylene glycol Transcutol RTM
Dye	For characterization study	Rhodamine-123 Rhodamine red FluorescenceIsothiocyanate (FITC) 6- Carboxy fluorescence
Alcohol	For providing the softness for vesicle membrane As a penetration enhancer	Ethanol Isopropyl alcohol
Cholesterol	For providing the stability to vesicle membrane	Cholesterol

Effectiveness of high alcohol content

Ethanol is an established efficient permeation enhancer [41,42]and is present in quite high concentration (20-50%) in ethosomes. However, due to the interdigitation effect of ethanol on

lipid bilayers, it was commonly believed that vesicles could not coexist with a high concentration of ethanol [43].

Touitou[4] discovered and investigated lipid vesicular systems embodying ethanol in relatively high concentration and named them ethosomes. The basic difference between liposomes and ethosomes lies in their composition. The synergistic effect of a combination of the relatively high concentration of ethanol (20-50%) in vesicular form in ethosomes was suggested to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration, the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids [45,46]. In addition, the vesicular nature of ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The versatility of ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like acyclovir[47], minoxidil[48], trihexyphenidyl[49], testosterone [50], cannabidiol[51], and zidovudine[52].

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Ethosomes Skin Permeation Mechanism

The stratum corneum lipid multilayers at physiological temperature are densely packed and highly conformationally ordered. Ethosomal formulations contain ethanol in their composition that interacts with lipid molecules in the polar head group regions, resulting in an increased fluidity of the SC lipids. The high alcohol content is also expected to partial extract the SC lipids. These processes are responsible for increasing inter and intracellular permeability of ethosomes. In addition, ethanol imparts flexibility to the ethosomal membrane that shall facilitate their skin permeation. The interdigitated, malleable ethosome vesicles can forge paths in the disordered SC and finally release a drug in the deep layers of skin. The transdermal absorption of drugs could then result from the fusion of ethosomes with skin lipids. This is expected to result in drug release at various points along the penetration pathway[53-56].

This can be summarised into main pathways

1. Ethanol effect

2. Ethosomes effect

Ethanol effect

Ethanol acts as a penetration enhancer through the skin. Its penetration enhancing effect is well known. Ethanol interacts with lipid molecules in the polar hard group region, resulting in a reducing the rigidity of the stratum corneum lipids, increasing their fluidity. The intercalation of ethanol into the polar head group environment can result in an increase in the membrane permeability. In addition to the effect of ethanol on stratum corneum structure, the ethosome itself may interact with the stratum corneum barrier. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane [6,49]

Ethosomes effect

Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. In the case of ethosomes encapsulating drugs, the higher positive zeta potential imparted by the drug can improve skin attachment of the vesicles. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into a deep layer of skin[57,49].

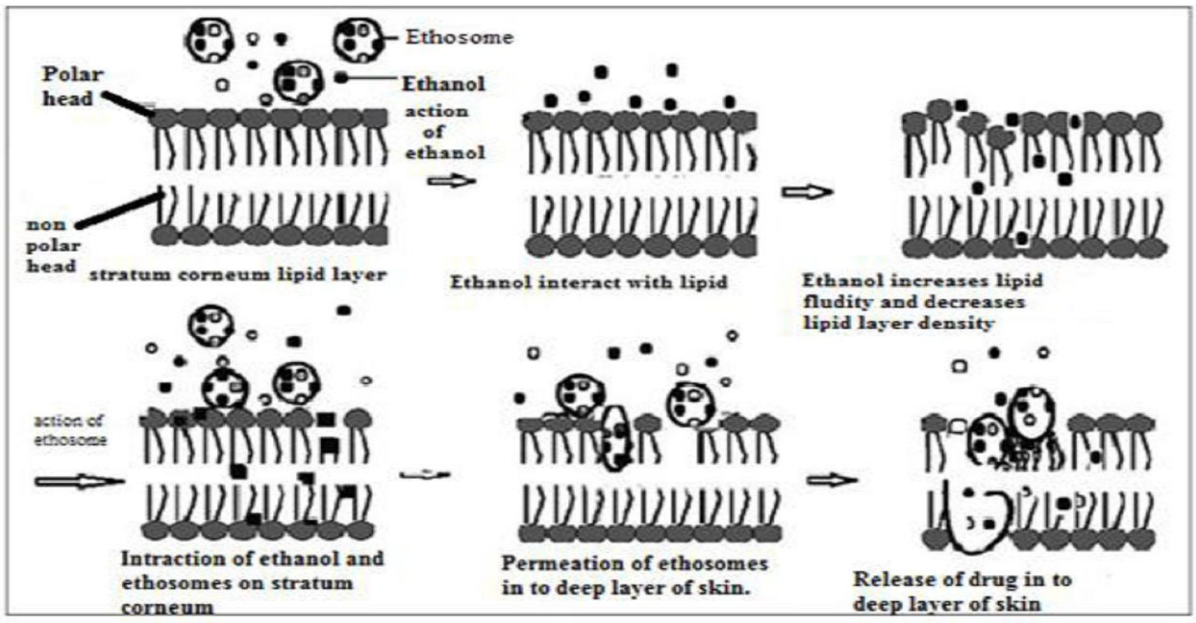

Figure 2: Proposed mechanism of action of ethosomes[56,25]

Table 2: Various drug molecules used in Ethosomal drug delivery[24,27,6]

Drug	Application	Results
Acyclovir	Treatment of Herpetic infection.	Î skin permeation Improved biological activity two to three times Improved in Pharmacodynamic profile
Minoxidil	Treatment of baldness	Accumulation in skin increased significantly
Insulin	Treatment of diabetes	Significant decrease in blood glucose level Provide control release
Anti-HIV agents Zidovudine Lamivudine	Treatment of AIDS	Improved transdermal flux Improved in biological activity two to three times Prolonging drug action Reduced drug toxicity Affected the normal histology of skin
Trihexyphenidyl hydrochloride	Treatment of Parkinsonian syndrome	Improved transdermal flu Provide controlled release Improved patient compliance Biologically active at dose several times lower than the currently used formulation
Antibiotic Cannabidiol Erythromycin	Prevents inflammation and edema Efficient healing of S. aureus induced deep dermal infections	Improved skin deposition Improved biological activity Prolonging drug action
Bacitracin	Treatment of dermal infections	Reason for ethosomal formulation
Azelaic acid		Poor skin permeation
Ammonium glycyrrhizinate		Pilosebaceous targeting
NSAIDS (Diclofenac)		
DNA		
Testosterone	Treatment of male hypogonadism	
Salbutamol .	Anti-asthmatic Bronchodilator	
Cyclosporine ·	Treatment of Inflammatory skin disease	

Mechanisms of preparation of Ethosomes

There are four methods which can be used for the formulation and preparation of ethosomes. They are simple and convenient and do not involve any sophisticated instrument nor complicated processes.

1. Cold method

2. Hot method

3. Classic method

4. Mechanical dispersion method

Cold Method

This method is most commonly used for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of the mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 30ºC in a water bath. The water heated to 30ºC in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of an ethosomal formulation can be decreased to desire extend using sonication[58] or extrusion[56] method.Finally, the formulation is stored under refrigeration [52,45,59,60].

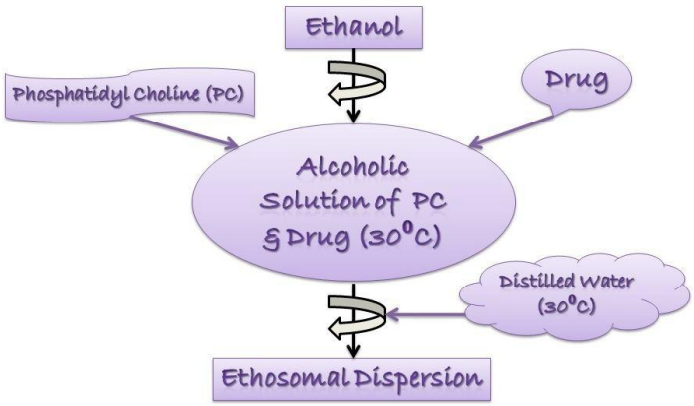

Fig 3: Ethosomal preparation by Cold Method[5]

Hot method

In this method, phospholipid is dispersed in water by heating in a water bath at 40ºC until a colloidal solution is obtained. In a separate vessel, ethanol and propylene glycol are mixed and heated to 40ºC. Once both mixtures reach 40ºC, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties [45,61, 62]. The vesicle size of an ethosomal formulation can be decreased to the desired extent using probe sonication or extrusion method.

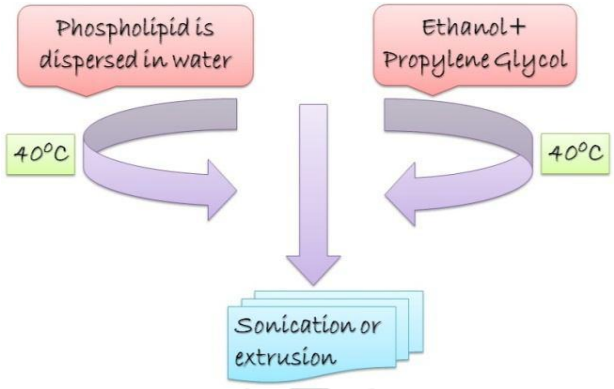

Figure 4: Ethosomal preparation by Hot Method[5]

Classic method

The phospholipid and drug are dissolved in ethanol and heated to 30°C±1°C in a water bath. Double distilled water is added in a fine stream to the lipid mixture, with constant stirring at 700 rpm, in a closed vessel. The resulting vesicle suspension is homogenized by passing through a polycarbonate membrane using a hand extruder for three cycles[60,63,62.]

Mechanical dispersion method

Soya phosphatidylcholine is dissolved in a mixture of chloroform: methanol in the round bottom flask (RBF). The organic solvents are removed using rotary vacuum evaporator above lipid transition temperature to form a thin lipid film on the wall of the RBF. Finally, traces of solvent mixture are removed from the deposited lipid film by leaving the contents under vacuum overnight. Hydration is done with different concentration of hydroethanolic mixture containing drug by rotating the RBF at the suitable temperature (Dubey et al. 2007)[64,65].

Characterization of Ethosomal Formulations

Vesicle shape (morphology)[66,67]

Transmission electron microscopy

Scanning electron microscopy

Entrapment efficiency[68,69,1]

Minicolumn centrifugation method

Fluorescence spectrophotometry, Ultracentrifugation technique

Vesicle size and size distribution[70]

Dynamic light scattering method

Photon correlation spectroscopy (PCS).

Vesicle Skin interaction study

Confocal laser scanning microscopy-Depth of penetration from ethosomes can be visualized by confocal laser scanning microscopy (CLSM) (Dayan and Touitou, 2002)[50].

Fluorescence microscopy

Transmission electron microscopy

Eosin-Hematoxylin staining [71,72, 6]

Phospholipid-ethanol interaction

31P NMR

Differential scanning calorimeter[50,73,1].

Degree of deformability

Degree of deformability of the ethosomal preparation can be performed by extrusion method[74, 75]

Zeta potential

Zeta potential is an important parameter that affects the aggregation of vesicles and depicts the physical stability of vesicular systems and it can be measured by Zeta meter[76] (Rao et al 2008).

Turbidity

Nephelometer (Cevc et al. 1995)[77,78]

***In-vitro* drug release study**

Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion[77,51]

Drug deposition study

Franz diffusion cell[74,75]

Stability study

The ability of ethosomal formulations to retain the drug was checked by keeping the preparations at different temperatures, i.e. 25±2°C (room temperature), 37±2°C and 45±2°Cfor different periods of time. The stability of ethosomes can also be determined quantitatively by monitoring size and morphology of the vesicles using DLS and TEM (Toll et al 2004)[79].

Surface tension activity measurement

Ring method in a Du Nouy ring tensiometer(Cevc et al .2004)[80]

APPLICATIONS OF ETHESOMES

Cosmeceutical and Therapeutic and applications

Cosmeceutical Applications of Ethosomes

The usage of ethosomes in cosmeceuticals offer many advantages including, increase the stability of the cosmetic chemicals, decrease skin irritation from the irritating cosmetic chemicals, and also for transdermal permeation enhancement[60].

Compositions and size of the vesicles main factors to be considered to obtain these advantages of the elastic vesicles for cosmeceutical applications. Topical administration of many antioxidants is one of the effective approaches to diminish oxidative injury in the skin for cosmetic and cosmeceutical applications.

Antioxidants, however, are usually not stable and can be degraded by exposing to light. These antioxidants include vitamin E, vitamin C, and flavonoids. Vitamin E is one of the major exogenous lipophilic antioxidants, usually found in tissues. Its topical application can enhance the skin protection from exogenous oxidants. Addition of vitamin E to cosmetics and many dermatological products found to decrease the production of lipid peroxides in the epidermis as well as to protect against UV exposure and some destructive chemicals and physical agents.

In 2008Koli et al., formulated 'Anti-oxidant Ethosomes for Topical Delivery Utilizing the Synergistic Properties of Vitamin A Palmitate, Vitamin E, and Vitamin C,' and the evaluation revealed that the synergistic interaction of Vitamin C in the aqueous core and Vitamin A and E in the lipid bilayer, provide complete protection from the oxidation of the ethosome formulations. This has suggested that although elastic and non-elastic liposomes are not beneficial for the delivery of α-tocopherol through the skin, the entrapment of the vitamin either in elastic or non-elastic liposomes can increase its photo-stability under UVB irradiation[81].

In 2004 Esposito et al.,[82]prepared a topical vehicle (gel) of ethosomes and liposomes entrapped with azelaic acid (Anti-keratinizing agent used in the treatment of acne) the result demonstrated that ETHOS 40 could be responsible for a higher azelaic acid, with respect to ETHOS 20 and liposomes.

Recently, Osmotics Inc., an American company reported a new cellulite cream called liposuction, formulated by ethosomal technology that penetrated the skin lipid barrier and delivered ingredients directly into the fat cells. Ingredients in liposuction improved the appearance of cellulite by up to 80% in less than 60 days. (Verma and Pathak, 2010)[56].

Therapeutic Applications of Ethosomes

Ethosomes can be used for various purposes in drug delivery. Mainly they used as a replacement of liposomes. Ethosomes can be used for the transdermal delivery of hydrophilic and impermeable drugs through the skin.

Transdermal Delivery of Hormones

Delivery of ant arthritic drug

Delivery of antipsoriatic and antineoplastic agent

Delivery of Atopic Dermatitis

Delivery NSAIDS

Pilosebaceous Targeting

Delivery of DNA

Transcellular delivery

Delivery of antibiotics

Delivery of Anti-Viral Drugs

Transdermal delivery of challenging drugs

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Transdermal Delivery of Hormones

The oral route is the commonest and convenient route of drug administration, however, administration of hormones presents with problems like high first-pass metabolism, low oral bioavailability and several dose-dependent side effects like virilization, acne, and gynecomastia .these side effects reduce patient compliance increasing the risk of treatment failure [83].

Insulin usual delivery is invasive due to its git associated problems. Thus non-invasive delivery of proteins is a better option for overcoming the problems associated with oral delivery [84-85].

In 1999, Dkeidek and Touitou[86] investigated the in-vivo effect of ethosomal insulin formulation in lowering blood glucose levels using normal and diabetic SDI rats. A Hill Top patch containing insulin ethosomes was applied on the abdominal area of an overnight fated rat.it was observed that that insulin delivered from this patch produced a significant decrease (up to 60%) in BGL in both normal and diabetic rats. While insulin application from a control formulation was not able to reduce the BGL.

In 2000 Touitouet al[87]conducted a comparative permeability study of testosterone ethosomes with a marketed transdermal patch of testosterone (Testoderm® patch, Alza Corporation, California) across rabbit pinna skin. It was observed that the ethosomal formulation had about 30-times higher skin permeation compared to that marketed formulation. With significantly improved pharmacokinetic parameters like AUC and Cmax of ethosomalTestosome as compared to Testoderm®.

Delivery of anti-parkinsonism agent

1n 2001, Dayan and Touitou,[88] prepared and compared an ethosomal formulation of the psychoactive drug trihexyphenidyl hydrochloride (THP) and compared its delivery with that of a classical liposomal formulation for the treatment of Parkinson's disease. THP is an M1 muscarinic receptors antagonist used in the treatment of Parkinson disease. It has a short biological half-life (3 hours) and oral administration is difficult due to motor disorders and neurological manifestations associated with a parkinsonian syndrome. THP ethosomal formulation, when visualized under the transmission and scanning electron microscopes, were viewed as small phospholipid vesicles. The value of the transdermal flux of THP through nude mouse skin from ethosomes was 87-, 51-, and 4.5-times higher than that from liposome, phosphate buffer, and hydroethanolic solution, respectively. The quantity of THP remaining in the skin at the end of 18 hours was significantly higher after the application of ethosomes than after the application of liposome or hydroethanolic solution (control). These results indicated the better skin permeation potential of ethosomal-THP formulation and its use for the better management of Parkinson disease.

Delivery of ant arthritic drug

In 2003 Lodzki et al., [51] prepared Cannabidiol CBD-ethosomal formulation for transdermal delivery for the treatment of rheumatoid arthritis. Skin deposition results showed significant accumulation of Cannabidiol (CBD) in the skin and underlying muscles after application of

the CBD-ethosomal formulation to the abdomen of Mice. A plasma concentration study showed that a steady state level was reached in 24 hours, which was maintained through 72 hours. A significant increase in biological anti-inflammatory activity of CBD-ethosomal formulation was observed when tested by using the carrageenan-induced rat paw edema model. it was concluded that ethosomal encapsulation of CBD significantly increased its skin permeation, accumulation, and hence, its biological activity.

Recently in 2010, Barupal et al.[90] Preparation and Characterized Ethosomes for Topical delivery of Aceclofenac and compared its activity to a marketed gel preparation. Aceclofenac is mainly used for the treatment of rheumatoid arthritis and osteoarthritis[2]. In some comparative studies of joint diseases, there was a tendency for aceclofenac to be better tolerated than diclofenac or ketoprofen with fewer patients being withdrawn from treatment due to gastric intolerance [3]. The patient treated with oral administration of aceclofenac suffers from various side effects, such as gastrointestinal ulcer and anemia due to gastrointestinal bleeding. Transdermal application of aceclofenac, as an alternative route of administration has demonstrated better to sort-out these problems. The data obtained suggest the possibility of substituting systemic treatment of osteoarthritis and rheumatoid arthritis with local treatment. This would result in decreased gastrointestinal associated side-effects, thereby potentially increasing patient compliance.

Manish et al.,2011[3]. Prepared Nano-size dethosomes bearing ketoprofen for improved transdermal delivery.Vesicle sizes varied from120.376.1 to 410.2721.8 nm depending on the concentrations of soya phosphatidylcholine(SPC) and ethanol. Entrapment efficiency increased with concentrations of SP Cand ethanol. The formulations exhibitd entrapment efficiencies of 42–78%. *In vitro* release through cellophane membrane showed sustained release of drug from ethosomal permeation across human skin revealed improved drug permeation and higher transdermal flux with ethosomal formulations compared to hydroethanolic drug solution. Kinetics of *in vitro* skin permeation showed zero order drug release from formulations. Based on *in vitro* transdermal flux, the estimated steady state *in vivo* plasma concentration from ethosomes attained therapeutic drug levels whereas hydroalcoholic drug solution exhibited subtherapeutic drug concentration with a patch size of 50cm^2. Skin permeation of ethosomal formulations assessed by confocal microscopy revealed enhanced permeation of Rhodamine123 loaded formulation in comparison to the hydroalcoholic solution.

In 2013 Fan et al[92] prepared enhanced topical delivery of tetrandrine by ethosomes for treatment of arthritis. The purpose of this work was to explore the feasibility of ethosomes for improving the antiarthritic efficacy of tetrandrine by topical application. It was found that tetrandrine was a weak base (pK(a) = 7.06) with pH-dependent partition coefficient. *Ex-vivo* permeation and deposition behavior demonstrated that the drug flux across rat skin and deposition of the drug in rat skin for ethosomes was 2.1- and 1.7-fold higher than that of liposomes, respectively. Confocal laser scanning microscopy confirmed that ethosomes could enhance the topical delivery of the drug in terms of depth and quantity compared with liposomes. The ethosomes were shown to generate substantial enhancement of therapeutic efficacy of tetrandrine on Freund's complete adjuvant-induced arthritis with regard to liposomes. These results indicated that ethosomes would be a promising career for topical delivery of tetrandrine into and across the skin.

Delivery of Anti-Viral Drugs

In 2007 Mishra et al., reported ethosomes for transcutaneous immunization. Antigen-loaded ethosomes for transcutaneous immunization against Hepatitis B were prepared and characterized, they showed greater entrapment efficiency, optimal size range, and a unilamellar, spherical shape compared to conventional liposomes. Spectral bio-imaging and flow cytometric studies showed an efficient uptake by murine dendritic cells *in-vitro*, reaching a peak by 180 minutes. Using human cadaver skin, the transcutaneous delivery potential of the antigen-loaded antigen ethosomes demonstrated a much higher skin permeation of the antigen in comparison to the conventional liposomes and soluble antigen preparation. The topically applied HBsAg-loaded ethosomes in mice showed a robust systemic and mucosal humoral immune response compared to the intramuscularly administered alum-adsorbed HBsAg suspension, the topically applied plain HBsAg solution, and the hydroethanolic (25%) HBsAg solution. HBsAg-loaded ethosomes are able to generate a protective immune response and their ability to transverse and target the immunological milieu of the skin finds a potential application in the development of a transcutaneous vaccine against Hepatitis B virus[93].

Zidovudine is a potent antiviral agent acting on acquired immunodeficiency virus. Oral administration of zidovudine is associated with strong side effects. Therefore, an adequate zero order delivery of zidovudine is desired to maintain expected anti-AIDS effect[94,95].

The optimized ethosomal formulation exhibited a transdermal flux of 78.5±2.5 mg/cm2/h across rat skin, while the hydroethanolic solution gave a flux of only 5.2±0.5 mg/cm2/h of zidovudine.

The flux from ethanolic solution was found to be 7.2±0.6 mg/cm2/h. Jain et al[52]concluded from this study that ethosomes could increase the transdermal flux, prolong the release and present an attractive route for sustained delivery of zidovudine.

Acyclovir is another anti-viral drug that widely used topically for treatment of Herpes labialis[96-97].The conventional marketed acyclovir external formulation is associated with poor skin penetration of hydrophilic acyclovir to dermal layer resulting in weak therapeutic efficacy[98]. It is reported that the replication of virus takes place in the basal dermis [99-101].

To overcome the problem associated with the conventional topical preparation of acyclovir, Horwitz et al. [47] formulated the acyclovir ethosomal formulation for dermal delivery. They have clinically evaluated its performance in a double-blind, randomized study with a marketed formulation of acyclovir (Zovirax, Glaxo-Wellcome) in terms of time to crust formation, time to loss of crust and proportions of lesions not progress beyond the popular stage (abortive lesions). Significant improvement in all evaluated clinical parameters was observed when the disorder was treated with the ethosomal formulation in comparison to the marketed formulation. The average time to crusting of lesions was 1.6 vs 4.3 days in the parallel arm and 1.8 vs. 3.5 days in the crossover arm ($P<0.025$) for ethosomal acyclovir and Zovirax, respectively. Hence, shorter healing time and the higher percentage of abortive lesions were observed when acyclovir was loaded into ethosomes.

Delivery of antipsoriatic and antineoplastic agent

Dubey et al. 2007 evaluated methotrexate an antipsoriatic, anti-neoplastic, highly hydrosoluble agent with limited transdermal permeation.They developed optimized ethosomes-loaded methotrexate and the skin permeation of the developed formulation revealed an enhanced permeation of rhodamine red loaded formulation to the deeper layers of the skin. The formulation retained its penetration power after storage and the vesicle skin interaction study also highlighted the penetration enhancing an effect of ethosomes, with some visual penetration pathways and corneocyte swelling[64].

Delivery of Atopic Dermatitis

In 2012 Li et al prepared and evaluated tacrolimus-loaded ethosomes: physicochemical characterization and *in-vivo* evaluation.Tacrolimus, an immunosuppressant for treating atopic dermatitis (AD), they investigated inhibition action upon allergic reactions of mice aiming at improving pharmacological effect for tacrolimus in that commercial tacrolimus ointment (Protopic®) with poor penetration capability exhibited weak impact on AD compared with common glucocorticoid.

Results showed that the ethosomes showed lower vesicle size and higher encapsulation efficiency (EE) as compared with traditional liposomes with cholesterol. Additionally, the quantity of tacrolimus remaining in the epidermis at the end of the 24-h experiment was statistically significantly greater from the ethosomal delivery system than from commercial ointment (Protopic®) ($p<0.01$), suggesting the greater penetration ability to the deep strata of the skin for ethosomes. Interestingly, tacrolimus-loaded ethosomes with ethanol, in contrast to that with propylene glycol, showed relatively higher penetration activity except for insignificant differences in EE and polydispersity index. Topical application of ethosomal tacrolimus displayed the lowest ear swelling in BALB/c mice model induced by repeated topical application of 2,4-dinitrofluorobenzene compared to traditional liposomes and commercial ointment and effectively impeded accumulation of mast cells in the ear of the mice, suggesting efficient suppression for the allergic reactions. They concluded that the ethosomal tacrolimus delivery systems may be a promising candidate for topical delivery of tacrolimus in the treatment of AD[102].

Pilosebaceous targeting

In 2004, Maiden et al., prepared and evaluated the minoxidil ethosomal formulation. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness. The conventional topical formulation has very poor skin permeation and retention properties. It was reported that the quantity of minoxidil accumulated into nude mice skin after application of its ethosomal formulation was 2.0-, 7.0-, and 5.0-fold higher when compared to ethanolic phospholipid dispersion, hydroethanolic solution, an ethanolic solution of the drug, each containing 0.5% of the drug. These results indicated the possibility of using ethosomes for pilosebaceous targeting of minoxidil to achieve better clinical efficacy[103].

Topical Delivery of DNA

The skin has evolved into an excellent protective barrier to various environmental pathogens, it is immunologically active and thus able to express the genes. In relation to the aforementioned facts a potential application of ethosomes, is in the topical delivery of DNA molecules, for expression of genes in the skin cells. In 2003 Touitou et al encapsulated the GFP-CMV-driven transfecting construct into the ethosomal formulation. They applied this formulation to the dorsal skin of five-week-old, male CD-1 nude mice for 48 hours. 48 hours later, the treated skin was removed and penetration of green fluorescent protein (GFP) formulation was observed by Confocal laser scanning microscopy (CLSM). It was observed that the topically applied ethosome-GFP-CMV-driven transfecting construct enabled efficient delivery and expression of genes in the skin cells. Thus it was suggested that ethosomes could be used as carriers for gene therapy applications that required transient expression of genes. These results also showed the possibility of using ethosomes for effective transdermal immunization[104].

In 2004 Gupta et al.,[105]reported the immunization potential of using transfersomal formulation. Thus, better skin permeation ability of ethosomes opens the possibility of using these dosage forms for the delivery of immunizing agents.

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Delivery of Antibiotics

Generally, oral administration of antibiotics causes several allergic reactions along with a number of untoward side effects.Topical delivery of antibiotics is, therefore, a better option for increasing the therapeutic efficacy of these agents. Conventional external preparations possess low permeability to deep skin layers and subdermal tissues[106].

Ethosomes can address this problem by delivering sufficient quantity of antibiotic into deeper layers of skin. Ethosomes penetrate rapidly through the epidermis and bring an appreciable amount of drugs into the deeper layer of skin and suppress infection at their root.

Godin and Touitou[88,107]prepared bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery. CLSM experiments revealed that ethosomes facilitated the co-penetration of antibiotic and phospholipid into cultured 3T3 Swiss albino mice fibroblasts. The results obtained by CLSM experiment were confirmed by FACS techniques and it was found that ethosomes penetrated the cellular membrane and released the entrapped

drug molecules within the cells. The results of this study showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy.

Transdermal delivery of challenging drugs

Additional studies on improved transdermal delivery by ethosomes have been cited in the literature. 1n 2005 Paolino et al.,[108] evaluated the potential application of ethosomes for dermal delivery of ammonium glycyrrhizinate. Ammonium is useful for the treatment of various inflammatory based skin diseases. *In-vitro* skin permeation experiments have shown that a significantly higher cumulative amount of drug has permeated from ethosomes (63.2%) than from the hydroalcoholic solution (22.3%) and the aqueous solution (8.9%) of ammonium glycyrrhizinate.Ethosomal formulation showed a very good skin tolerability in human volunteers for 48-hour application. Biological anti-edema activity was also significantly enhanced in case of an ethosomal formulation as compared to an ethanolic or aqueous solution of the drug.

Marketed products of ethosomal technology

From 2000, when Touitou et al., discovered ethosomes commercialization of the technology began.Only two companies which developed ethosome products (Verma and Pathak, 2010)

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Table 3: Marketed products of ethosomal technology [20,62]

Manufacturer	Name of product	Uses
Hampden Health, USA	Cellutight EF	Topical cellulite cream contains a powerful combination of ingredients to increase metabolism and break down fat
Genome Cosmetics, Pennsylvania	Decorin cream	Anti-aging cream, treating, repairing, and delaying the visible aging signs of the skin including wrinkle lines, sagging, age spots, loss of elasticity, and hyperpigmentation
Sinere, Germany	Nanominox	First minoxidil containing a product, which uses thosomes. Contains 4% Minoxidil, well-known hair growth promoter that must be metabolized by sulfation to the active compound
Novel Therapeutic Technologies, Israel	Noicellex	Topical anti-cellulite cream
orange peel Physonics, Nottingham, UK	Skin Genuity	Powerful cellulite buster reduces
Trima, Israel	Supravir cream	For the treatment of herpes virus, formulation of acyclovir drug has a long shelf life with no stability problems, stable for at least three years, at 25°C. Skin permeation experiments showed that the cream retained its initial penetration enhancing properties even after three years

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RECENT STUDIES CITED IN LITERATURE ON ETHOSOMAL TECHNOLOGY FOR THE PAST 5 YEARS

In 2013 Bhosale and Avachat Designed and developed ethosomal transdermal drug delivery system of valsartan with preclinical assessment in Wistar albino rats.

Valsartan (VLT) is a highly selective and orally active antihypertensive drug. However, its oral administration is associated with drawbacks like low bioavailability. VLT ethosomes were prepared by the cold method. VLT ethosomes were characterized by scanning electron microscopy. The prepared ethanolic liposomes were characterized to be spherical having the low polydispersity of nano-size range with good entrapment efficiency. ETC5 ethosomal suspension with 4% of phospholipon 90H and 40% of ethanol was found to have highest entrapment efficiency, i.e. $80.230 \pm 0.8748\%$. The permeation study of ethosomes was evaluated by *ex-vivo* diffusion study through rat abdominal skin using Franz's diffusion cells and the ETC5 ethosomal suspension was found to have the highest permeation with flux of 92.819 ± 1.539 µg/cm²/h when compared to the permeation profiles of drug solutions either in water or in a water-ethanol mixture. Transdermal application of ethosomal VLT on Wistar

rats showed better and prolonged antihypertensive activity in comparison to orally administered VLT suspension by virtue of transdermal permeation through Wistar rat skin. Histopathological study of skin applied with ETC5 showed intercellular permeation across the skin by dissolving intercellular lipids in epidermis without causing any rigorous changes in the skin cellular structure. In conclusion, ethosomes enabled the transdermal permeation of VLT, which amply proves its superiority over oral administration for antihypertensive treatment[109].

In 2014 Sarwa et al conducted Penetration studies of tamoxifen citrate loaded ethosomes and liposomes across human skin: a comparative study with confocal laser scanning microscopy. Ethosomal and liposomal formulations containing tamoxifen citrate were prepared and evaluated for their penetration properties in human cadaver skin using Franz diffusion cell and confocal laser scanning microscope (CLSM). The results clearly showed that ethosomal vesicles showed a better drug permeation profile than that of liposomal vesicles. In addition, the low fluorescence intensity in CLSM was recorded with liposomes as compared to ethosomes, indicating a lower cumulative amount of drug permeation from liposomal vesicles. Furthermore, CLSM showed uniform fluorescence intensity across the entire depth of skin in ethosomal treatment, indicating high penetrability of ethosomal vesicles through human cadaver skin. In contrast, the low penetrability of conventional liposomal vesicles was recorded as penetration was limited to the upper epidermis layer of skin as evident from visualization of intact liposomal vesicles in CLSM[110].

Zhai et al in 2015 prepared ethosomes for skin delivery of ropivacaine: preparation, characterization and *ex-vivo* penetration properties.

Ropivacaine, a novel long-acting local anesthetic, has been proved to own superior advantage. However, Naropin® Injection, the applied form in a clinic, can cause patient non-convenience. The RPV-loaded ethosomes were prepared with thin-film dispersion technique and the results showed that optimized RPV-ethosomes displayed a typical lipid bilayer structure with a narrow size distribution of 73.86 ± 2.40 nm and drug loading of 8.27 ± 0.37%, EE of 68.92 ± 0.29%. The results of DSC and XRD study indicated that RPV was in an amorphous state when encapsulated into ethosomes. In addition, the results of *ex-vivo* permeation study proved that RPV-ethosomes could promote the permeability in a high-efficient, rapid way (349.0 ± 11.5 μg cm(-2) at 12 h and 178.8 ± 7.1 μg cm(-2) at 0.5 h). The outcomes of histopathology study forecasted that the interaction between ethosomes and skin

could loosen the tight conjugation of corneocyte layers and weaken the permeation barrier. In conclusion, RPV-ethosomes could be a promising delivery system to encapsulate RPV and deliver RPV for transdermal administration[111].

Shen et al in 2015 [112] prepared and evaluated Compound antimalarial ethosomal cataplasm: preparation, evaluation, and mechanism of penetration enhancement.Malaria is a serious public health problem in some parts of the world. The problems of recurrence and drug resistance are increasingly more serious. Artesunate-loaded ethosomes and febrifugine-loaded ethosomes were prepared, and their characteristics evaluated. Drug-loaded ethosomes were incorporated in the matrix of cataplasm to form the compound antimalarial ethosomal cataplasm. With the help of ethosomal technology, the accumulated permeation quantity of artesunate significantly increased at 8 hours after administration, which was 1.57 times as much as that of conventional cataplasm. Soon after administration, the ethosomal cataplasm could make a large quantity of antimalarial drugs quickly penetrate through the skin, then the remaining drug in the ethosomal cataplasm could be steadily released. These characteristics of ethosomal cataplasm are favorable for antimalarial drugs to kill Plasmodium spp. quickly and prevent the resurgence of Plasmodium spp. The ethosomal cataplasm showed good antimalarial efficiency. The negative conversion rates were 100% and the recurrence rates were 0% at all dosages. Data obtained in this study showed that the application of ethosomal technology to antimalarial cataplasm could improve the transdermal delivery of a drug, enhance the efficacy, and facilitate practical application in the clinic.

In 2015 Babaie et al prepared and evaluated lidocaine loaded nanoliposomes. Results showed that the particle size, zeta potential, EE and LC of the optimum formulation were 105.4 ± 7.9 nm, -33.6 ± 2.4 mV, 40.14 ± 2.5 %, and 8.02 ± 0.71 respectively. SEM results confirmed the non-aggregated nano-scale size of prepared nanoethosomes. The particle size of ethosomes and EE of Lidocaine were depended on the phospholipid and ethanol concentrations. XRD results demonstrated the drug encapsulation in amorphous status interpreting the achieved high drug EE and LC values. *In vitro* and *in vivo* assays confirmed the appropriate skin penetration of Lidocaine with the aid of nanoethosomes and existence of deposition of nanoethosomes in deep skin layers, respectively, they concluded that the developed nanoethosomes are a potential and suitable carrier for topical delivery of anesthetics like Lidocaine[113].

In 2016 Simões et al. prepared ethosomes for enhanced skin delivery of griseofulvin. Griseofulvin (GRF) is an important antifungal drug with low bioavailability and, for this reason, a topical formulation with a targeted action and minimal systemic effects appears to be a preferable solution. GRF poor solubility has limited the development of topical formulations and their release to the market. GRF vesicles had a mean size of 130nm. Permeation and penetration assays revealed that GRF-loaded ethosomes have an adequate profile to be used in a topical formulation since drug retention in the stratum corneum was achieved. Cell viability tests proved this formulation presented no cytotoxicity to HaCaT cells for concentrations below 50μg/mL. The skin diffusion test evidenced the potential of the developed formulation to target skin dermatophytes. The results obtained in this study contribute to a new perspective in the topical treatment of fungal infections[114].

Khan and Wong in 2016[115]prepared Microwave-aided skin drug penetration and retention of 5-fluorouracil-loaded ethosomes.

They investigated the interplay effects of ethosomes and microwave on transdermal drug delivery. Skin pre-treatment by microwave and applied with liquefied medicine is deemed to 'cement' the skin thereby raising skin drug deposition.

Data obtained showed that the skin drug retention was promoted using larger ethosomes with negative zeta potentials that repelled anionic lipids of skin and hindered vesicle permeation into deep layers. These ethosomes had low ethanol content. They were less able to fluidize the lipid and fluidize the protein domains in epidermis to enlarge aqueous pores for drug permeation. Pre-treatment of the skin by 2450 MHz microwave for 2.5 min further increased skin drug penetration and retention of low ethanol ethosomes and provided lower drug permeation than cases treated for 1.15 min and 5 min. A 2.5 min treatment might be accompanied by specific dermal protein fluidization via C=O moiety which translated to macromolecular swelling, narrowing of intercellular spaces at lower skin layers, increased drug retention, and reduced drug permeation.it was concluded that ethosomes and microwave synergized to promote skin drug retention.

In 2016 Garg et al prepared nanosized ethosomes-based hydrogel formulations of methoxsalen for enhanced topical delivery against vitiligo: formulation optimization, *in-vitro* evaluation and preclinical assessment. Ethosomes were found to be spherical and multilamellar in structures having nanometric size range with narrow size distribution and

high encapsulation efficiency. Ethosomal formulations showed significant skin permeation and accumulation in the epidermal and dermal layers. The fluorescence microscopy study using 123 Rhodamine exhibited enhanced permeation of the drug-loaded ethosomes in the deeper layers of skin. Also, the developed formulation showed insignificant phototoxicity and erythema vis-à-vis the conventional cream. The results were cross-validated using a histopathological examination of skin segments. They concluded that the ethosomes-based hydrogel formulation was found to be a promising drug delivery system demonstrating enhanced percutaneous penetration of methoxsalen with reduced phototoxicity and erythema, leading to improved patient compliance for the treatment against vitiligo[116].

Limsuwan et al., 2017[117] prepared and evaluated ethosomes of Phenylethyl Resorcinol as Vesicular Delivery System for Skin Lightening Applications

Ethosome formulations containing phenyl ethyl resorcinol (PR) were developed. The formulation was produced from 0.5% w/v PR, 0.5% w/v cholesterol from lanolin, 3% w/v L-α-phosphatidylcholine from soybean, 30% v/v absolute ethanol, and water up to 100% v/v. It was characterized by a vesicular size of 389 nm, a low polydispersity index of 0.266, a zeta potential of -34.19 ± 0.44 mV, high PR entrapment efficiency of 71%, and good stability on storage at 4 and 30°C at 75% RH for 4 months. *In vitro* studies using pig skin revealed that permeation coefficient of PR from ethosomes was significantly higher than that from liposomes. *In vitro* retention profiles showed that PR accumulation in pig skin following application of ethosome formulations was 7.4-, 3.3-, and 1.8-fold higher than that achieved using liposomes, 20% propylene glycol solution, and 30% hydroethanolic solution, respectively. An inhibition value of around 80% was measured for the antityrosinase activity of PR in pigskin. Additionally, ethosomes exhibited higher tyrosinase inhibition activity and melanin content reduction when compared to other formulations in B16 melanoma cells. Ethosomes did not cause acute dermal irritation in albino rabbits. This data demonstrated that ethosomes are capable of delivering PR into the skin efficiently and hold promise for topical application of skin lightening products.

Furthermore, Yang et al., 2017[118] investigated mechanism of transdermal permeation promotion of lipophilic drugs by ethosomes

Ethosomes can promote the penetration of lipophilic drugs into the skin, but the underlying mechanism is still unknown. The formulation of ethosomes was optimized using the Box-

Behnken experimental design, in which Rhodamine B and 1-palmitoyl-2-{12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl}-sn-glycero-3-phosphocholine were used to simulate a model lipophilic drug and act as a fluorescent tracer of ethosomal phospholipids, respectively. Liposomes with the same phospholipid concentration and a hydroethanolic solution with the same ethanol concentration were also prepared as controls. The percutaneous progression of the above fluorescent preparations was observed by confocal laser scanning microscopy, and the fluorescence intensity of the images was analyzed. The optimized ethosome formulation consisted of 2.45% yolk phospholipids, 30% ethanol, and 67.55% distilled water. The percutaneous permeation of Rhodamine B in the optimized ethosomes was superior to that in hydroethanolic solution ($P<0.05$) and liposomes ($P<0.05$). The ethosomes could penetrate the skin via the percutaneous pathway of the hair follicle and stratum corneum, while during the process of penetration, the vesicles were broken and the phospholipids were retained in the upper epidermis, with the test compounds penetrating gradually. The superior percutaneous penetration of ethosomes was linked to the synergistic effects of their ingredients. The percutaneous pathways of ethosomes included open hair follicles and stratum corneum pathways. In addition, the vesicles might break up during percutaneous penetration in the superficial layer of the skin, allowing the test compounds to keep permeating into the deeper layer alone, while the phospholipid was retained in the upper epidermis.

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CONCLUSION

Ethosomes are soft, malleable vesicles characterized by simplicity in their preparation, safety, and efficacy of therapeutic agents. They offer a good opportunity for the non-invasive delivery of small, medium and large sized drug molecules. This conclusion is supported by results from the first clinical study of acyclovir-ethosomal formulation providing better skin permeation than liposomes or hydro-alcoholic solution targeting deeper skin layers for various skin diseases. Their ability to encapsulate hydrophilic drugs, cationic drugs, proteins, and peptides has opened new challenges and opportunities for the development of novel improved therapies on the market. Research in this area will allow better control over drug release *in vivo* Improving therapeutic outcome. Therefore it can be a logically concluded that ethosomal technology possesses a promising future ineffective transdermal delivery of therapeutic agents**.**

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