REVIEW ARTICLE

TOPICAL DELIVERY OF DRUGS USING ETHOSOMES: A REVIEW

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ABSTRACT

The skin is the largest organ of the human body that restricts the movement of drug to the systemic circulation. Topical drug delivery system is a system where the drug reaches the systemic circulation through the protective layer i.e. skin. The main disadvantage of this route is the low diffusion rate of the drugs which is across the stratum corneum layer of the skin. To overcome this problem to a certain extent, ethosomal delivery for drugs and herbal compounds has been recently introduced. Literature studies indicate that ethosomal formulation of acyclovir shows high therapeutic efficiency with shorter healing time in the treatment of recurrent herpes labialis than conventional Zovirax. Also, the ethosomes of minoxidil enhanced the skin permeation of minoxidil *in vitro* in comparison to its ethanolic or phospholipid ethanolic micellar solution or hydroethanolic solution. The advantages of this system include increased drug permeation, increased drug entrapment and improved drug delivery. Ethosomal drug delivery system opens up doors for the development of new and novel therapies for treating male pattern baldness, as it is an easier way to prepare, in addition to its safety and efficacy. In this review article, we have focused on methods of preparation of ethosomes, characterization techniques, applications, details about the various research trials for the management of androgenic alopecia and various ethosomal products in market.

Keywords: ethosomes, androgenic alopecia, topical route, skin, ethanol.

INTRODUCTION

Topical route of delivery system is a non-invasive method that enables the drug to reach the systemic circulation. From the pharmaceutical point of view, topical drug delivery offer advantages compared with other routes of administration, including avoidance of first-pass metabolism, lower administration frequency, smaller fluctuations in plasma drug profile and good patient compliance[1]. Skin is a multilayered structure composed of stratum corneum (sc) the outermost and the tightest layer of skin and below it lies the epidermis and dermis[2]. Skin is a highly hydrophobic layer composed of differentiated non-nucleated cells and corneocytes, which are filled with keratins and embedded in the lipid domain. Since the rate limiting step for skin absorption of most molecules is considered to be because of this non-viable layer, percutaneous permeation of molecules is believed to be governed by diffusion laws. The skin contributes to 4% of the total body weight. The extent of skin permeation of a compound may depend on the route of administration. The barrier nature of skin makes it difficult for most drugs to penetrate into and permeate through it[3]. In order to improve topical drug delivery, many kinds of techniques, including complex physical enhancement strategies, such as iontophoresis[4], sonophoresis[5], microneedle[6], and electroporation[7], and lipid vesicular systems such as emulsions[8], microemulsions, and liposomal-based delivery systems, have been used to overcome the barrier of SC. Amongst these, liposomal-based delivery systems, including conventional liposomes[9], ultraflexible liposomes[10] and ethosomes[11-14] offer a promising strategy for improving skin drug delivery and have attracted much interest in recent years due to the merits, including convenience for use and harmlessness to skin.

There are three pathways which are involved in the transdermal permeation of drugs: (1) through the

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intercellular lipid zone in sub cutaneous (SC) region; (2) through the skin appendages; and (3) through the keratin bundles in SC[1].

Alopecia mean loss of hair from areas where hair normally grows. It comes in a variety of patterns in a variety of cases. Male pattern baldness or androgenetic alopecia is the most common form. Aesthetic treatment options include wigs, hairpieces, and surgical transplantation. The latter is expensive. Other options include low-level laser light therapy. There is no sufficient evidence for these or other miscellaneous options as mainstream treatments. Hair loss concealers are cosmetic sprays, powders and creams that are applied to the hair and scalp to either make the hair appear thicker or to reduce the hair fall or to improve the hair growth in the scalp.

Minoxidil and finasteride are the two drugs currently approved by the United States Food and Drug Administration (USFDA) for the treatment and management of androgenetic alopecia or male pattern baldness. In general, for the treatment, oral administration of finasteride tablets is the usual practice. But it often causes side effects like male infertility and erectile dysfunction. Different research investication have been done on both minoxidil as well as finasteride. Few of the research investigations with their merits and limitations are mentioned in Table I and Table II.

There is a need for novel delivery vehicles for the drugs/ herbal compounds that would provide superior drug loading and skin targeting following topical application. Many strategies have been tried by different authors using the carriers micro/nanoemulsions, liposomes, polymeric encapsulation and other lipophilic carriers. Ethosomes, a novel type of liposomes containing 20–50% of ethanol (V/V), have the potential to fulfill these therapeutic requirements because they can enhance drug loading by increasing solubility and transdermal absorption. Ethosomes, representing a new, innovative and interesting vesicular system, have been used for transdermal drug delivery in recent years because they can enable a drug to pass through the skin and increase the accumulation of drug in the skin. Elasticity of ethosome vesicles is considered to increase in presence of ethanol and it also helps in the passage through the lipid pores and channels in the stratum corneum is facilitated[15,16]. Drug release from ethosomes is expected to be influenced by fusion of vesicle membranes with skin lipids at various points along the penetration pathway[17]. *In vitro* and *in vivo*, animal and clinical studies have shown that ethosomes are efficient at improving dermal/transdermal delivery of both hydrophilic and lipophilic moieties[18,19]. Ethosomes are reported to improve the skin delivery of various drugs[20]. Topical formulation of ethosomes with the drug/ herbal compounds for male pattern baldness or androgenetic alopecia for maximum permeability into the scalp skin can be explored with ethosomes. In the following sections, details pertaining to ethosomes for the application of topical drug delivery are discussed.

Ethosomes

Ethosomes are a modified form from liposomes, which have proved to be good carriers in the transdermal area. Ethosomes are mainly lipid vesicles made of phospholipids, ethanol, and water. They have an aqueous core which contains the ethanolic solution of drug and

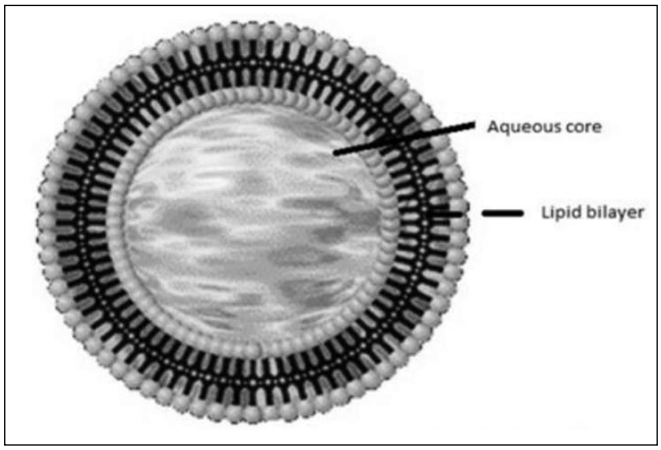

Fig. 1 Structure of Ethosomes (Source: pharmatutor.org)

the outer layer comprises of lipid bilayer (Fig. 1). The effect of ethanol fluidizing the bilayers of phospholipids contributes to the creation of vesicles with a malleable structure which enables to get molecules (drugs, pharmaceuticals, or active agents) to deeper layers of the skin[21,22]. Drug delivery from liposomes has limited results in the transdermal formulations due to unstable nature and low permeability[23-26]. Due to the concern owing to the stability of liposomes, a novel vesicular carrier, niosomes, was developed to counter the problems of low stability[27]. Despite that, liposomes and niosomes could not counter the problems of poor skin permeability[28]. Hence, ethanolic vesicles have been developed to enhance the permeation of drugs across the skin. The size range of ethosomes ranges from tens of nanometres to microns and the transdermal flux of ethosomes is more, besides its skin permeability[22,29,30]. Ethosomal systems are classified based on the composition into three types.

Classical ethosomes

In transdermal drug delivery, ethosomes were reported to be superior over classical liposomes because

Table I: Research trials carried out with Finasteride

Sr. No.	STUDY TITLE	MERITS	LIMITATIONS
1.	Finasteride in the treatment of men with androgenic alopecia[32].	In men with male pattern hair loss, finasteride 1mg/day slowed the progression of hair loss and increased hair growth in clinical trials over 2 years.	Patients reported adverse events related to sexual function and also adverse events on breast enlargement which reversed on discontinuation of drug.
2.	Changes in hair weight in men with androgenetic alopecia after treatment with finasteride 1 mg daily for 3 and 4 years[33].	Long-term finasteride treatment led to sustained improvement in hair weight compared with placebo. Hair weight increased to a larger extent than hair count, implying that factors other than the number of hairs, such as increased growth rate (length) and thickness of hairs, contribute to the beneficial effects of finasteride in treated men.	This study was extended from its original 48-week duration to nearly 4 years, the sample size available for evaluation decreased with time.
3.	Finasteride is the main inhibitor of 5-α reductase in dermal papillae of human hair follicles[34].	The study resulted in the conclusion that finasteride inhibited approximately 80% of 5-α reductase activity in the dermal papillae and connective tissue sheaths.	No specific limitations cited in the literature
4.	Preparation of novel polymeric microspheres for controlled release of finasteride[35].	The results showed that the mean diameter of microspheres was approx 2 mm and had both smooth and spherical surfaces. Greater encapsulation efficiency was obtained by increasing the ratio of polymer: finasteride.	No specific limitations cited in the literature
5.	Design and *in vitro*-evaluation of finasteride- loaded liquid crystalline nanoparticles for topical delivery[36].	Formulation with lower monoolein lipid exhibited higher skin permeation with a flux rate of 0.061±0.005 µg in 24 h.	The release profile was significantly altered with addition of different additives.
6.	Pharmacodynamic of P-3074 (finasteride 0.25% topical solution) in subjects with androgenetic alopecia[37].	Topical application and oral finasteride results found to be similar.	Only a 7- day dose of topical P-3074 solution was administered & therefore no long-term measurement of effectiveness of P-3074.
7.	Efficacy and safety of 3% minoxidil (MNX) versus combined 3% minoxidil / 0.1% finasteride (MFX) in male pattern hair loss : a randomised double blind , comparative study[38].	Although change of hair counts was not statistically different between two groups, global photographic assessment showed significantly greater improvement in MFX group than the MNX group.	There was no sexual side effect.
8.	Comparing the therapeutic effects of finasteride gel and tablet in treatment of the androgenetic alopecia[39].	The results of this study showed that the therapeutic effects of both finasteride gel and finasteride tablet were relatively similar to each other.	Local finasteride gel caused erythma in affected site which subsided on discontinuation. Decreasing libido due to tablet was also reported.
9.	Finasteride cream in hirsutism[40].	In this study of women with facial hirsutism, topically applied finasteride significantly decreased hair growth and thickness.	Cannot be used during pregnancy period. No other sexual side effects were found.

Table II: Research trials carried out with Minoxidil

Sr. No.	STUDY TITLE	MERITS	LIMITATIONS
1.	A multicellular randomized, placebo-controlled, double-blind clinical trial of a novel formulation of 5% minoxidil (MTF) topical form versus placebo in the treatment of androgenetic alopecia[41].	There was a significant increase in i) Hair counts in the 5% MTF group versus placebo(P<0.001) ii) Subjective assessment of improved hair loss condition in 5% MTF (P<0.001) group versus placebo. The 5% MTF was well tolerated over a 52 week period.	There was no collection of efficacy data beyond 16 weeks.
2.	Topical products for human hair regeneration: A comparative study on an animal model[42].	Results suggest that 2% minoxidil topical application is more efficacious than aminexilor or kerium in inducing hair growth as assessed by trichoscopy, hair weight examination & morphometric assessment. This study validates the hair growth effect of minoxidil & also demonstrates that kerium treatment induces good hair regrowth in Wistar rats.	The results failed to demonstrate significant variation in skin thickness between control and treated area in any of the four experimental groups.
3.	Effectiveness of 5% of minoxidil in treating male pattern baldness[43].	At the end of four months the 5% minoxidil solution judged very effective in stimulating new hair growth by 7.5%, effective by 55%, moderately effective by 31.3% of the men.	The 5% minoxidil solution was found ineffective by 6.2% of men. Skin related side effects were reported by 13 patients.
4.	A randomized evaluator blinded study of effect of microneedling in androgenetic alopecia: A pilot study[44].	Dermaroller along with minoxidil treated group was statistically superior to minoxidil treated group in promoting hair growth in men with Androgenic alopecia for all 3 primary efficacy measures of hair growth. In the microneedling group, 41(82%) patients reported more than 50% improvement versus only 2(4.5%) patients in the minoxidil group.	Issues regarding microneedling viz., different sizes of needles of the dermaroller, frequency, duration and end point of the procedure are yet to be answered.
5.	Double-blind, placebo-controlled evaluation of topical minoxidil in extensive alopecia areata[45].	Hair growth was seen in seven of eleven evaluable subjects (63.6%) in the minoxidil group and in five of fourteen evaluable subjects (35.7%) in the placebo group. Excellent, cosmetically acceptable hair growth was seen in three of eleven minoxidil-treated subjects (27.3%) and in one of fourteen placebo-treated subjects (7.1%).	Three instances of scalp itching and dermatitis was observed, two of which necessitated discontinuing the medication.
6.	3% topical minoxidil compared with placebo for the treatment of chronic severe alopecia areata[46].	Transient regrowth of sparse vellus hair occurred in twelve patients , bilaterally in eight, but it was not significant in any.	No significant side effects were noted, except for a moderately severe bilateral dermatatitis in one patient.
7.	Evaluation of oral minoxidil in the treatment of alopecia areata[47].	Oral minoxidil (5 mg every 12 hours), a dose demonstrated to be relatively well tolerated if a 2g sodium diet is strictly followed, was given to 65 patients with severe, treatment-resistant alopecia areata in an attempt to bypass the limitations of topical treatment and increase efficacy.	Although hair regrowth progressed more rapidly and was more extensive with oral than topical 5% minoxidil, cosmetic response was seen only in 18% of the patients. Neither serum nor tissue levels of minoxidil correlated with response.

Table III: Different additives used in formulation of Ethosomes

Sr. No.	Class	Example	Uses
1	Phospholipids	Soya phosphotidyl choline, Egg phosphotidyl choline, Dipalmityl phosphotidyl choline, Disteryl phosphotidyl choline	Vesicles forming agent
2	Polygylcol	Propylene glycol, transcutol	Skin penetration enhancer
3	Alcohol	Ethanol, isopropyl alcohol	Penetration enhancer, provides softness for vesicle membrane
4	Cholesterol	Cholesterol	Provide stability to vesicle membrane
5	Surfactant	Stearylamine	Improve solubility of poorly soluble drugs and also act as charge inducing agent.

Table IV: Various ethosome based products available in the market

Sr. No.	Name of Product	Manufacturer	Uses
1.	Supravir cream	Trima, Israel	For the treatment of herpes virus, formulation of acyclovir drug has a long shelf life with no stability problems, stable for 3 yrs, at 25°C. Skin permeation experiments showed that the cream retained its initial penetration enhancing properties even after 3 years.
2.	Noicellex	Novel Therapeutic Technologies, Israel	Topical anti-cellulite cream
3.	Cellutight EF	Hampden Health, USA	Topical cellulite cream, contains a powerful combination of ingredients to increase metabolism and break down fat.
4.	Nanominox	Sinere, Germany	Minoxidil 4% containing product, for hair growth
5.	Decorin cream	Genome Cosmetics, Pennsylvania, US	Anti – ageing cream that reduces the visible ageing signs of skin which includes wrinkle lines, sagging, age spots, hyper pigmentation.

they were smaller and had negative ζ-potential and higher entrapment efficiency. Moreover, classical ethosomes showed better skin permeation and stability profiles compared to classical liposomes[24,31,48]. The molecular weights of drugs entrapped in classical ethosomes have ranged from 130.077 Da to 24 kDa[49,50].

Binary ethosomes

Binary ethosomes were introduced by Zhou *et al.*[51] Basically, they were developed by adding another type of alcohol to the classical ethosomes. The most commonly used alcohols in binary ethosomes are propylene glycol (PG) and isopropyl alcohol (IPA)[52-56].

Methods of ethosome preparation

Ethosomes are usually prepared by the following methods[29,57,58].

Cold method

This is the most widely used method for the preparation of ethosomes. In the cold method, phospholipid (and any other lipid materials) and drug are dissolved in ethanol (and mixture of glycols) at room temperature. This mixture is then mildly heated to 30°C. Simultaneously, water is heated (to the same temperature as that of the ethanolic mixture) and added to the ethanolic mixture and stirred for some time. The vesicle size of the ethosomes can be decreased by sonication or extrusion of the ethanolic mixture[2]. During the whole process, care should be taken to prevent evaporation of ethanol. Finally, the formulations are stored under refrigeration. The flow sheet of the cold method is represented in Fig. 2.

Hot method

In this method, initially the phospholipids are dispersed in water and heated to 40°C. Simultaneously, ethanol is heated to the same temperature as that of the aqueous phase. The drug is dissolved either in water or ethanol depending on its properties propylene glycol has also been used. The organic phase is added to the aqueous phase and sonicated to reduce particle size[29,48,59]. A brief description of the hot method to prepare ethosomes is shown in Fig. 3.

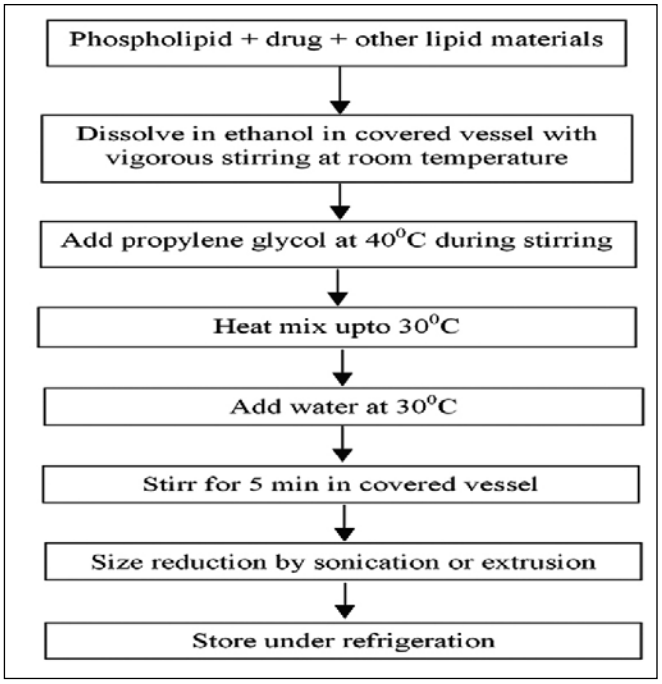

Fig. 2: Cold method for the preparation of ethosomes

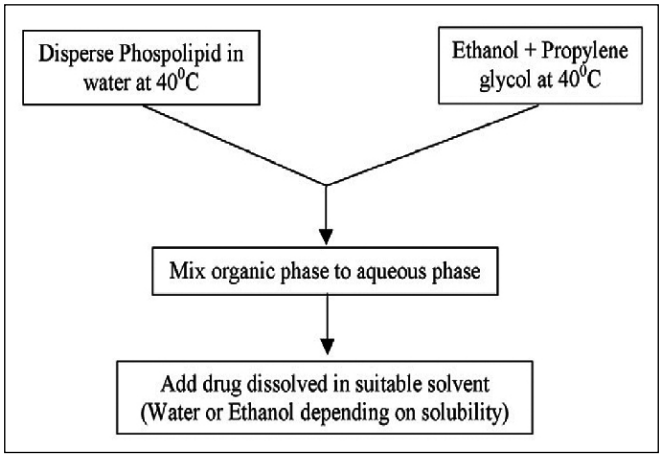

Fig. 3: Hot method for the preparation of ethosomes

Classic Mechanical Dispersion Method

Soya phosphotidylcholine is dissolved in a mixture of chloroform: methanol (3:1) in a round bottom flask. The organic solvents are removed using rotary vacuum evaporator above lipid transition temperature to form of a thin lipid film on wall of the flask. 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 flask at suitable temperature[60,61].

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[62].

CHARACTERIZATION OF ETHOSOMES

Visualization of the vesicles

The morphology of the ethosomes can be visualized using transmission electron microscopy (TEM) and scanning electron microscopy (SEM)[22].

Vesicle size and Zeta potential

The particle size and zeta potential of the ethosomes can be measured by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS)[22].

Entrapment efficiency

The entrapment efficiency of the ethosomes can be measured using ultra centrifugation method. The formulation is centrifuged at high speeds and the free drug present in the supernatant is estimated using a suitable analytical method[63].

Transition Temperature

The transition temperature, melting point and enthalpy of the ethosomes can be measured using differential scanning calorimetry (DSC)[64].

Vesicle Stability

The stability of the ethosomal vesicles can be observed by periodically assessing the vesicle size and structure of the vesicles over a period of time using DLS and TEM[65].

Penetration and permeation studies

The depth of penetration of ethosomes upon application on the skin can be visualized and measured by confocal laser scanning microscope. Ethsomes loaded with a dye can be applied on an animal skin and the depth of the penetration can be observed[66].

Uniqueness of ethosome

Ethosomes have been studied for the delivery of different drugs ranging from low molecular weight drugs like testosterone, minoxidil, acyclovir, trihexyphenidyl

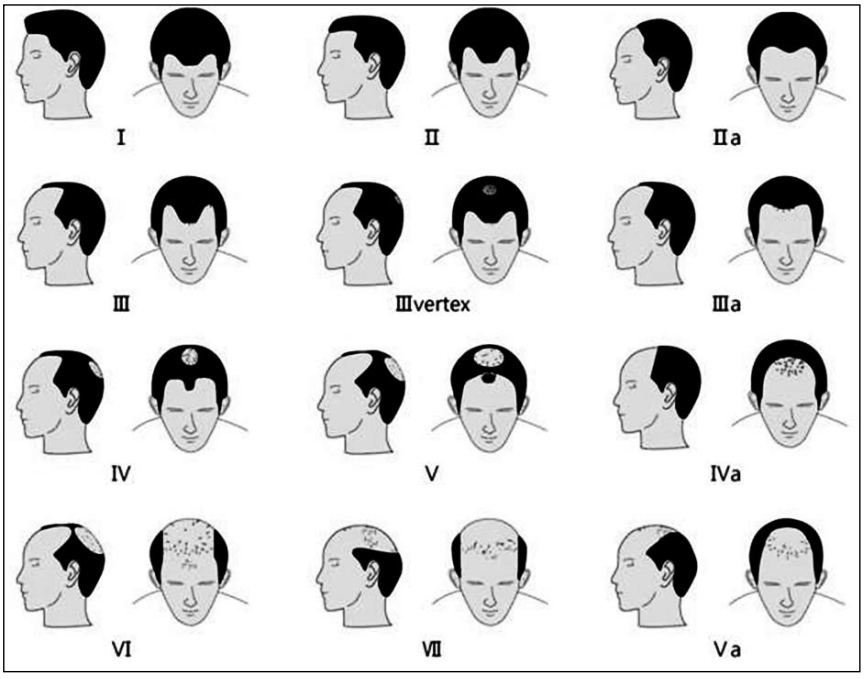

Fig. 4: Norwoods Classification for Androgenetic Alopecia or Male Pattern Baldness (Source : www.thewestminsterpractise.com)

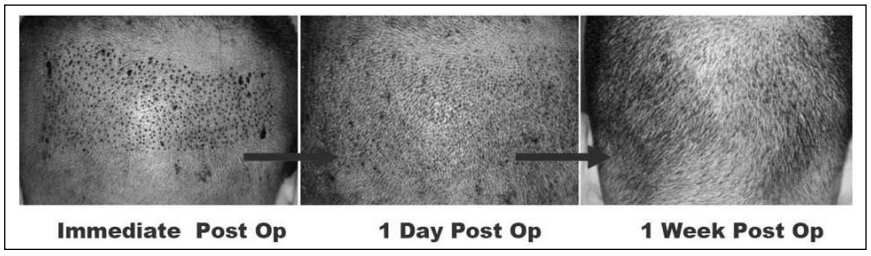

Fig. 5: Post hair transplant surgery (Source : www.regrowhair.com)

hydrochloride to high molecular weight polypeptides. They are also widely used for other clinical purpos like gene therapy, prostate cancer and hirsutism. Ethosomes are found to be productive in terms of delivery of anti-HIV/AIDS drugs via the transdermal route[67]. The ethanol content in ethosomes is considered to be higher[68]. The high content of ethanol in ethosomes helps in increased incorporation of drug in system as well as better penetrability of drug into the systemic circulation. Due to the interdigitation effect of ethanol on lipid bilayers, usage of high amount of ethanol would damage liposomal formulations. However, ethosomes with higher amount of ethanol (25-40%) have been formulated for better permeation of drug[69]. The different additives used in the formulation of ethosomes are given in Table III.

Mechanism of action of ethosomes

The permeation of ethosomes across the skin is mainly due to the effect of ethanol and ethosomes[70]. After the application of ethosomes on the skin, the ethanol penetrates into the intercellular lipids by rupturing them. This decreases the density of the lipids multilayer and also simultaneously increases the fluidity of the cell membrane lipids which will help in the permeation of the ethosomes across the skin. The release of the drug in the deep layers of the skin and its transdermal absorption could also be due to the result of a fusion of ethosomes, with skin lipids and drug release at various points along the penetration pathway[71]. Thus, ethosomes enhance the permeation of the drugs across the skin.

Advantages of ethosomes

1. Ethosomes cause enhanced permeation of drug through skin for transdermal and dermal delivery[72].
2. Peptides and protein molecules of large and diverse groups of drugs can be delivered using ethosomes.
3. Components used in ethosomes are safe and approved for pharmaceutical and cosmetic use.
4. No large-scale drug technology development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature.
5. Compared to iontophoresis and phonophoresis, the ethosomal drug administrated in semisolid form (gel or cream), leads to high patient compliance. In contrast, the former are relatively complicated to use, which will affect patient compliance.
6. High market attractiveness for products with proprietary technology. No complicated technical investments required for production of ethosomes as simple manufacturing techniques are available
7. The Ethosomal system is non-invasive and is available for immediate commercialization.
8. Various application in pharmaceutical, veterinary, and cosmetic fields.

Stability of ethosomes

Stability of the formulations was evaluated in terms of the entrapment capacity and the particle size for a specified period. The proper choice of the lipid composition

is an important factor in obtaining stable ethosomes dispersions with optimum pharmaceutical and therapeutic characteristics. In case of liposomes, upon storage, many different changes could occur.

The absence of electrostatic repulsion is likely to account for the tendency of the neutral liposome to aggregate, but in case of ethosomes, ethanol causes a modification of the net charge of the system and confers it some degree of steric stabilization as leading to increased stability of the dispersion against agglomeration that may also lead to a decrease in the mean vesicle size. Natural and / or synthetic phospholipid derived sources are used the lipid components for ethosomes. Phospholipids containing unsaturated fatty acids are known to undergo oxidative reactions which can cause permeability changes in the ethosomes bilayers due to the reaction products. Oxidative degradation of the lipids in general can be minimized by protecting the lipid preparation from light, by adding antioxidants such as α-tocopherol. Furthermore, hydrolysis of lipids leads to the formation of lyso-PC. The presence of lyso-PC enhances the permeability of ethosomes, and thus, it is essential to keep its level to a minimum in a given preparation[73, 74].

Marketed products of ethosomes

Ethosome was first invented by and the patent for this was awarded to Prof Elka Touitou along with her students at the Department of Pharmaceutics of Hebrew University[29]. Another formulation, Lipoduction TM, is being currently used in the treatment of cellulite. It contains pure grape extracts that acts as anti-oxidant and is marketed by USA. The different marketed products of ethosomes are summarised in Table IV. These products were produced a few years ago, but currently there is no detailed information available[75-77].

Research trials of ethosomes with herbal compounds

Topical application of herbal extracts/compounds containing using ethosomes are reported in literature. Rutin is a flavonoid that belongs to the class of flavonols. Rutin presents low skin permeation rate, i.e., a lack of ability to overcome the stratum corneum barrier, a property that could be considered an inconvenience to the efficacy of a cosmetic formulation to perform its antioxidant activity onto the skin was delivered using ethosomes and the results were encouraging[78,79]. Ascenso et al incorporated caffeine into ethosomes[54]. Permeation profiles of caffeine in the transethosomes permeated more, followed by ethosomes and transfersomes.

Li *et al*. associated tacrolimus with ethosomes and compared cutaneous permeation of the ethosomal vesicles with a commercially available ointment. Ethosomes developed greater penetration capacity in the deeper layers of the skin than the commercial ointment[80]. Paolino *et al*., incorporated ammonium glycyrrhizinate, a drug used in inflammatory skin diseases, in ethosomes[81].

Research trials of ethosomes in cosmetics

Koli *et al*., have formulated ethosomes to deliver vitamin E into the deeper layer of stratum corneum and the findings have 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[82].

In a study by Esposito *et al*., ethosomes and liposomes of azelaic acid (anti-keratinizing agent used in the treatment of acne) were prepared as a topical vehicle (gel) and the result demonstrated that ETHOS 40 could be responsible for a higher azelaic acid absorption with respect to ETHOS 20 and liposomes[83].

Therapeutic Application of Ethosomes

Dubey V *et al*., reported ethosomes for transcutaneous immunization. Antigen-loaded ethosomes for transcutaneous immunization against Hepatitis B were prepared and characterized, which showed greater entrapment efficiency, optimal size range, and a unilamellar, spherical shape in comparision to conventional liposomes. Spectral bio imaging and flow cytometric studies showed an efficient uptake of HBsAg-loaded ethosomes by murine dendritic cells *in vitro*, reaching a peak by 180 minutes. The study demonstrated a much higher skin permeation of the antigen in comparision to the conventional liposomes and soluble antigen preparation. HBsAg-loaded ethosomes topically applied 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. The protective immune response and their ability to transverse and target the immunological milieu with HBSAg-loaded ethosomes in skin finds a potential application in the development of a transcutaneous vaccine against Hepatitis B virus[61].

Touitou *et al*., compared the skin permeation potential of testosterone ethosomes (Testosome) across rabbit pinna skin, with the marketed transdermal patch of testosterone (Testoderm® patch, Alza corporation, California). The authors observed nearly 30 times higher

skin permeation of testosterone from the ethosomal formulation as compared to the marketed formulation. The AUC and Cmax of testosterone significantly improved after the application of Testosome as compared to Testoderm®. Hence, both *in vitro* and *in vivo* studies demonstrated improved skin permeation and bioavailability of testosterone from the ethosomal formulation. This group designed a testosterone non-patch formulation to reduce the area of application in their progressive study and found that with ethosomal testosterone formulation, the area of application required to produce the effective plasma concentration was 10 times less than that required by the commercial gel (AndroG, US) formulation[22].

Lodzki *et al.*, prepared the CBD-ethosomal formulation for transdermal delivery of cannabiol for the treatment of rheumatoid arthritis. The skin deposition results study showed significant accumulation of cannabidiol (CBD) in the skin, and underlying muscles after application of 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. The encapsulation of CBD in ethosomes significantly increased its skin permeation, accumulation, and hence, its biological activity[84].

Dayan and Touitou prepared ethosomal formulation of the psychoactive drug trihexyphenidyl hydrochloride (THP) and compared its delivery with that with the classical liposomal formulation for the treatment of Parkinsons disease. THP is an M1 muscarinic receptors antagonist and used in the treatment of Parkinson's disease. THP has a short biological half-life (3 hours) and its oral administration is difficult due to motor disorders and neurogical manifestations associated with Parkinsonian syndrome. THP ethosomal formulation, when visualized under transmission and scanning electron microscopes, were viewed as small phospholipid vesicles. The transdermal flux value of the 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's disease[85].

Dubey *et al.*, developed optimized ethosomes-loaded methotrexate and the skin permeation profile of the developed formulation revealed an enhanced permeation of rhodamine red loaded formulation to the deeper layers of the skin. After storage the formulation retained its penetration power and the vesicle skin interaction study also highlighted the penetration enhancing effect of ethosomes, with some visual penetration pathways and corneocyte swelling[61].

Paolino *et al.*, investigated 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 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 ethosomal formulation as compared to ethanolic or aqueous solution of the drug[81].

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Tunyaluk *et al.*, formulated ethosome containing phenylethyl resorcinol (PR). 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, low polydispersity index of 0.266, zeta potential of – 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 antityrosinase activity of PR in pig skin. Consistently, 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. These findings demonstrate that ethosomes are capable of delivering PR into the skin efficiently and hold promise for topical application of skin lightening products[86].

A. K. Barupal et al., prepared aceclofenac ethosomes with varying the quantity of ethanol 10-50% (v/v), lecithin 1-4% (w/V), propylene glycol 5-20% (V/V) and evaluated for their vesicle size, shape and surface morphology, entrapment efficiency and *in vitro* drug permeation study. Ethosomes of average size of 1.112 µm with a spherical shape bearing smooth surface were observed by transmission electron microscopy and surface electron microscopy. The maximum entrapment of ethosomes was 91.06±0.79%. Cumulative amount of drug permeated through the biological membrane was found to be in the range of 0.26±0.014 to 0.49±0.032 mg/cm^2 . Stability profile of prepared system was assessed for 45 days and the results revealed that very less degradation of drug was observed during storage condition[87].

Ethosomal systems for other applications

A hair dye of transethosomes was developed and found to be more efficient in delivering and enhancing the adsorption of black tea extracts to the hair surface than a hydroalcoholic solution of the same extract[88].

Based on a literature search, only seven research articles had reported ethosomal patch formulations for several drugs, ie, artesunate and febrifugine,[89] ligustrazine,[90, 91] valsartan,[92] tizanidine hydrochloride,[93] and insulin[94].

Two studies report the formulation of ethosomal creams. Both of these involved the incorporation of *Curcuma longa* extract-loaded ethosomal systems in a cream base as a photoprotective and antiwrinkle agent.[95,96] In both studies, *C. longa* extract-loaded ethosomal creams were applied to human volunteers and showed promising results as either a photoprotective[95] or an antiwrinkle agent[96].

Clinical trials

Based on literature searches, only three clinical trials have been conducted on ethosomal systems in human volunteers. Horwitz et al carried out a pilot, double-blind, randomized clinical study to compare the efficacy of an ethosomal acyclovir preparation and commercially available acyclovir cream (Zovirax®) in treating recurrent herpes labialis in 40 human volunteers. The results revealed that the ethosomal acyclovir preparation performed better than Zovirax cream and showed significant improvement in all the evaluated clinical parameters, such as the time of crust formation and disappearance and pain parameters[97]. The efficacy of ethosomal gel of clindamycin phosphate and salicylic acid was evaluated in a pilot clinical trial of 40 acne patients treated with the gel twice daily for 8 weeks. Volunteers treated with ethosomal gel showed considerable improvement in acne condition, with a decreased number of comedones, pustules, and total number of lesions compared to placebo[98]. Ethosomal preparation of prostaglandin E_1 was evaluated in a pilot clinical study in patients with erectile dysfunction. It was observed that 12 of 15 tested patients had improved peak systolic velocity and penile rigidity. Erection duration was 10–60 minutes[99]. There were no reported adverse skin reactions associated with the treatment in any of the aforementioned clinical trials.

Androgenetic alopecia

Male pattern hair loss, also commonly called as androgenic alopecia, is a type of hair loss observed due to the shrinkage of hair follicles because of the deposition of dihydrotestosterone. There are different stages in which hair loss takes place in scalp. This has been classified by Norwood (Fig. 4). Men with androgenic alopecia are reported to have lower amounts of total testosterone and higher amounts of unbound/ free testosterone, 5 - alpha reductase, and free androgens such as dihydrotestosterone. Additionally, the signaling pathway and crosstalk between the androgens promotes hair loss. In general, at the somatic stem cell level, androgens promote differentiation of facial hair dermal papillae to facilitate hair growth. But, in patients with androgenic alopecia, this process is inhibited at the scalp[100].

Different methodologies for treating androgenic alopecia

As hair loss is considered as a serious matter, many remedies have been evaluated to a great extent. Both males and females have issues regarding hair loss.

Hormones

The role of androgens in the etiology of androgenetic alopecia is considered to be a widespread aid in the treatment. Anti-androgens act primarily through blockade

of the androgen receptor at the hair follicles. These hormones are delivered systemically in women. This treatment is not advisable to men as it can promote development of feminine characteristics. Topical oestrogens and anti-oestrogens have been used in both men and women[101].

Surgery

Hair restoration surgery involves hair transplantation, scalp reduction surgery, or a combination of both (Fig. 5). Hair transplantation is considered to be less invasive. Follicles that are not affected by miniaturization are redistributed over the scalp under local anesthesia[101]. The result of hair transplantation is based on the texture of hair, the quality of hair, and also the number of transplanted hair in relation to the area to be covered or densified.

Combination therapies

Combination of surgical and medical therapy has been found to be effective than surgery alone. A study revealed better clinical results for male patients treated with combination of finasteride 1 mg daily and hair surgery versus male patients treated with hair surgery alone, 12 months after follicular unit transplantation[102].

Limitations of ethosomes

Loss of product while transferring from organic to water media is reported[103]. If shell locking is not proper; there are chances of coalescence and parting while transferring into water media with poor yield of the product[104]. Drugs requiring attainment of high concentration in the blood cannot be administered. Ethosomes can be limited only to potent molecules. It offers slow, sustained drug delivery. The molecular size of the drug should be considerably less so that it is absorbed percutaneously. It is not very economical.

Conclusion

Ethosomes have brought new understanding towards vesicular research for topical delivery, which may provide enhanced skin permeation with respect to that shown by liposomes. Ethosomes can be potential carriers for transportation of drugs across the scalp for treating androgenic alopecia and male pattern hair loss. Drugs like finasteride and minoxidil can be explored for topical applications using ethosome to overcome the conventional dosage form side effects, even the method of action is essentially unknown. Dutasteride, 5 alpha-reductase enzyme inhibitor, the androgen receptor antagonists spironolactone and cyproterone acetate and prostaglandin analogue latanoprost can also be explored using ethosomes as carrier at institutional level even though not approved by US FDA for treating male pattern baldness. The extracts (petroleum ether) of *Phyllanthus niruri*, by inhibiting 5α-reductase enzyme, have shown promising results for the treatment of testosterone-induced alopecia in an animal model. Similarly, the seed extracts of *Croton tiglum* purified by sodhana process using milk and the ethanolic extracts of the rhizomes of *Zingiber officianalis* were used in equal ratio for topical application as a paste for the treatment of alopecia areata. Ethosomes with drugs/herbal compounds can be investigated in depth for the development of topical formulation to manage male pattern baldness. Ethosomes can also be used for the encapsulation of proteins, cationic drugs, peptides, and hydrophilic drugs.

REFERENCES

1. Brown MB, Martin GP, Jones SA, Akomeah FK. Dermal and transdermal drug delivery systems: current and future prospects. **Drug Delivery**. 2006;13(3):175-87.
2. Verma DD, Fahr A. Synergistic penetration enhancement effect of ethanol and phospholipids on the topical delivery of cyclosporin a. **J. Control. Release.** 2004;97(1):55-66.
3. Maghraby GM, Williams AC, Barry BW. Skin hydration and possible shunt route penetration in controlled estradiol delivery from ultradeformable and standard liposomes. **J. Pharm. Pharmacol**. 2001;53(10):1311-22.
4. Essa EA, Bonner MC, Barry BW. Iontophoretic estradiol skin delivery and tritium exchange in ultradeformable liposomes. **Int. J. Pharm**. 2002;240(1-2):55-66.
5. Merino G, Kalia YN, Delgado-Charro MB, Potts RO, Guy RH. Frequency and thermal effects on the enhancement of transdermal transport by sonophoresis. **J. Control. Rel**. 2003;88(1):85-94.
6. J. Zahn JD, Trebotich D, Liepmann D. Microdialysis microneedles for continuous medical monitoring. **Biomed. Microdevices**. 2005;7(1):59-69.
7. Tokudome Y, Sugibayashi K. The synergic effects of various electrolytes and electroporation on the in vitro skin permeation of calcein. **J. Control. Rel.**. 2003;92(1-2):93-101.
8. Frelichowska J, Bolzinger MA, Valour JP, Mouaziz H, Pelletier J, Chevalier Y. Pickering w/o emulsions: drug release and topical delivery**. Int. J. Pharm.** 2009; 368 (1-2):7-15.
9. Mezei M, Gulasekharam V. Liposomes-a selective drug delivery system for the topical route of administration I. Lotion dosage form. **Life Sci.**, 1980;26(18):1473-7.
10. Chen M, Li XR, Zhou YX, Yang KW, Chen XW, Deng Q, Liu Y, Ren LJ. Improved absorption of salmon calcitonin by ultraflexible liposomes through intranasal delivery. **Peptides**. 2009;30(7):1288-95.
11. Paolino D, Celia C, Trapasso E, Cilurzo F, Fresta M. Paclitaxel-loaded ethosomes®: potential treatment of squamous cell carcinoma, a malignant transformation

of actinic keratoses. **Eur. J. Pharm. Biopharm**. 2012;81(1):102-12.

12. Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl: ethosomes vs. liposomes. **Biomaterials**. 2000;21(18):1879-85.
13. Dubey V, Mishra D, Nahar M, Jain V, Jain NK. Enhanced transdermal delivery of an anti-HIV agent via ethanolic liposomes. Nanomedicine: Nanotechnology, **Biol Med**. 2010;6(4):590-6.
14. Dubey V, Mishra D, Jain NK. Melatonin loaded ethanolic liposomes: physicochemical characterization and enhanced transdermal delivery. **Eur. J. Pharm. Biopharm**. 2007;67(2):398-405.
15. Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. **J. Adv. Pharm. Techn. Res.** 2010;1(3):274.
16. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. **AAPS Pharmscitech**. 2007;8(4):249.
17. Fang YP, Tsai YH, Wu PC, Huang YB. Comparison of 5-aminolevulinic acid-encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. **Int.J. Pharma**. 2008;356(1-2):144-52.
18. Elsayed MM, Abdallah OY, Naggar VF, Khalafallah NM. Deformable liposomes and ethosomes: mechanism of enhanced skin delivery. **Int. J. Pharm**. 2006; 322(1-2) : 60-6.
19. Biju SS, Talegaonkar S, Mishra PR, Khar RK. Vesicular systems: an overview. **Ind. J. Pharm. Sci**. 2006;68(2).
20. Bendas ER, Tadros MI. Enhanced transdermal delivery of salbutamol sulfate via ethosomes. **AAPS Pharmscitech**. 2007;8(4):213.
21. Godin B, Touitou E. Ethosomes: new prospects in transdermal delivery. **Crit Rev Ther Drug Carrier Syst.** 2003;20(1):63–102.
22. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. **J Control Rel**. 1999;65(3):403–18. 22.
23. Patel MC, Patel HK, Suthar RM, Patel SR. Liposomes: as a topical drug delivery system. **Int J Pharm Chem Sci**. 2012;1(1):1-0.
24. Hua S. Lipid-based nano-delivery systems for skin delivery of drugs and bioactives. **Frontiers pharmacol**. 2015 Sep 30;6:219.
25. Mezei M, Gulasekharam V. Liposomes - a selective drug delivery system for the topical route of administration I. Lotion dosage form. **Life Sci.** 1980; 26(18):1473-1477.
26. Yarosh DB. Liposomes in investigative dermatology. **Photodermatol. Photoimmunol. Photomed**. 2001; 17(5):203-212.
27. Deo MR, Sant VP, Parekh SR, Khopade AJ, Banakar U V. Proliposome-based transdermal delivery of levonorgestrel. **J. Biomater. Appl.** 1997;12(1):77-88.
28. Lasch J, Laub R, Wohlrab W. How deep do intact liposomes penetrate into human skin? **J. Control. Release.** 1992;18(1):55-58.
29. Touitou E I, Composition of applying active substance to or through the skin. US Pat 5 540 934, 1996, July 30.
30. Rakesh R, Anoop KR. Ethosomes for transdermal and topical drug delivery. **Int. J. Pharm. Pharm. Sci.** 2012;4(SUPPL.3):17-24.
31. Sarwa KK, Suresh PK, Rudrapal M, Verma VK. Penetration of tamoxifen citrate loaded ethosomes and liposomes across human skin: a comparative study with confocal laser scanning microscopy. **Curr. Drug Deliv.** 2014;11(3):332–337.
32. Jain S, Patel N, Madan P, Lin S. Quality by design approach for formulation, evaluation and statistical optimization of diclofenac-loaded ethosomes via transdermal route. **Pharm Dev Technol.** 2015;20(4):473–489.
33. Zhang Z, Wo Y, Zhang Y, *et al. In vitro* study of ethosome penetration in human skin and hypertrophic scar tissue. **Nanomed.,** 2012;8(6):1026–1033
34. Mishra D, Mishra PK, Dabadghao S, Dubey V, Nahar M, Jain NK. Comparative evaluation of hepatitis B surface antigen-loaded elastic liposomes and ethosomes for human dendritic cell uptake and immune response. **Nanomed.,** 2010;6(1):110–118.
35. Peng D, Huang K, Liu Y, Liu S. Preparation of novel polymeric microspheres for controlled release of finasteride. **Int. J. Pharm.** 2007;342(1-2):82-86.
36. Madheswaran T, Baskaran R, Thapa RK, et al. Design and In Vitro Evaluation of Finasteride-Loaded Liquid Crystalline Nanoparticles for Topical Delivery. **AAPS PharmSciTech**. 2013;14(1):45-52.
37. Caserini M, Radicioni M, Leuratti C, Terragni E, Iorizzo M, Palmieri R. Effects of a novel finasteride 0.25% topical solution on scalp and serum dihydrotestosterone in healthy men with androgenetic alopecia. **Int. J. Clin. Pharmacol. Ther.** 2016;54(01):19-27.
38. Tanglertsampan C. Efficacy and safety of 3% minoxidil versus combined 3% minoxidil / 0.1% finasteride in male pattern hair loss: A randomized, double-blind, comparative study. **J. Med. Assoc. Thail.** 2012;95(10):1312-1316.
39. Hajheydari Z, Akbari J, Saeedi M, Shokoohi L. Comparing the therapeutic effects of finasteride gel and tablet in treatment of the androgenetic alopecia. **Indian J. Dermatol. Venereol. Leprol.** 2009;75(1):47-51.
40. Lucas, MD, FACP, Face KJ. Finasteride Cream in Hirsutism. **Endocr. Pract.** 2001;7(1):5-10.
41. Olsen EA, Whiting D, Bergfeld W, et al. A multicenter, randomized, placebo-controlled, double-blind clinical trial of a novel formulation of 5% minoxidil topical foam versus placebo in the treatment of androgenetic alopecia in men. **J. Am. Acad. Dermatol.** 2007;57(5):767-774.
42. Orasan MS, Bolfa P, Coneac A, Muresan A, Mihu C. Topical products for human hair regeneration: A comparative study on an animal model. **Ann. Dermatol.** 2016;28(1):65-73.
43. Hasanzadeh H, Nasrollahi SA, Halavati N, Saberi M, Firooz A. Efficacy and safety of 5% minoxidil topical foam

in male pattern hair loss treatment and patient satisfaction. **Acta dermatovenerol. Alp.,** Pannonica, et Adriatica. 2016;25(3):41-4.

44. Dhurat R, Sukesh M, Avhad G, Dandale A, Pal A, Pund P. A randomized evaluator blinded study of effect of microneedling in androgenetic alopecia: A pilot study. **Int. J. Trichology.** 2013;5(1):6.
45. Price VH. Double-blind, placebo-controlled evaluation of topical minoxidil in extensive alopecia areata. **J. Am. Acad. Dermatol.** 1987;16(3):730-736.
46. Fransway AF, Muller SA. 3 Percent topical minoxidil compared with placebo for the treatment of chronic severe alopecia areata. **Cutis.** 1988;41(6):431-435.
47. Fiedler-Weiss VC, Rumsfield J, Buys CM, West DP, Wendrow A. Evaluation of oral minoxidil in the treatment of alopecia areata. **Arch. Dermatol.**, 1987;123(11):1488-90.
48. Jain S, Patel N, Madan P, Lin S. Quality by design approach for formulation, evaluation and statistical optimization of diclofenac-loaded ethosomes via transdermal route. **Pharm Dev Technol.** 2015;20(4):473–489.
49. Zhang Z, Wo Y, Zhang Y, et al. In vitro study of ethosome penetration in human skin and hypertrophic scar tissue. **Nanomed.,** 2012;8(6):1026–1033
50. Mishra D, Mishra PK, Dabadghao S, Dubey V, Nahar M, Jain NK. Comparative evaluation of hepatitis B surface antigen-loaded elastic liposomes and ethosomes for human dendritic cell uptake and immune response. **Nanomed.,** 2010;6(1):110–118.
51. Zhou Y, Wei Y, Liu H, Zhang G, Wu X. Preparation and in vitro evaluation of ethosomal total alkaloids of *Sophora alopecuroides* loaded by a transmembrane pH-gradient method. **AAPS PharmSciTech.** 2010;11(3):1350–1358
52. Li G, Fan Y, Fan C, et al. Tacrolimus-loaded ethosomes: physicochemical characterization and in vivo evaluation. **Eur. J. Pharm. Biopharm.,** 2012;82(1):49–57.
53. Zhang JP, Wei YH, Zhou Y, Li YQ, Wu XA. Ethosomes, binary ethosomes and transfersomes of terbinafine hydrochloride: a comparative study. **Arch. Pharm. Res.** 2012;35(1):109–117.
54. Akhtar N, Pathak K. Cavamax W7 composite ethosomal gel of clotrimazole for improved topical delivery: development and comparison with ethosomal gel. **AAPS PharmSciTech.** 2012;13(1):344–355.
55. Dave V, Kumar D, Lewis S, Paliwal S. Ethosome for enhanced transdermal drug delivery of aceclofenac. **Int. J. Drug Deliv.** 2010;2(1):81–92.
56. 40. Shen LN, Zhang YT, Wang Q, Xu L, Feng NP. Enhanced in vitro and in vivo skin deposition of apigenin delivered using ethosomes. **Int. J. Pharm.,** 2014;460(1–2):280–288.
57. Alkilani AZ, McCrudden MTC, Donnelly RF. Transdermal drug delivery: Innovative pharmaceutical developments based on disruption of the barrier properties of the stratum corneum. **Pharm.,** 2015;7(4):438-470.
58. Wilson V, Siram K, Rajendran S, Sankar V. Development and evaluation of finasteride loaded ethosomes for targeting to the pilosebaceous unit. **Artif. Cells, Nanomed. Biotechnol**. October 2017:1-10.
59. Bhalaria MK, Naik S, Mishra AN. Ethosomes: A novel system for antifungal drugs in the treatment of topical fungal disease. **Ind. J. Exp. Biol**., 2009;47:368–75.
60. Dubey V, Mishra D, Jain NK. Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. **Eur. J. Pharm. Biopharm**. 2007;67:398–405.
61. Dubey V, Mishra D, Dutta T, Nahar M, Saraf DK, Jain NK. Dermal and transdermal delivery of an anti-psoriatic agent via ethanolic liposomes. **J. Contr. Rel.,** 2007;123:148–54.
62. Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. **AAPS Pharm. Sci. Tech.** 2007;8:1–9.
63. Fry DW, White JC, Goldman ID. Rapid separation of low molecular weight solutes from liposomes without dilution. **Anal. Biochem.** 1978;90(2):809-815.
64. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, et al. Liposome: Classification, preparation, and applications. **Nanoscale Res. Lett.** 2013;8(1):1-8.
65. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume-Peytavi U. Penetration profile of microspheres in follicular targeting of terminal hair follicles. **J. Invest. Dermatol**. 2004;123(1):168-176.
66. López-Pinto JM, González-Rodríguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. **Int. J. Pharm.** 2005;298(1):1-12.
67. Gupta U, Jain NK. Non-polymeric nano-carriers in HIV/AIDS drug delivery and targeting. **Adv. Drug Deliv. Rev.** 2010;62(4-5):478-490.
68. Elsayed MMA, Abdallah OY, Naggar VF, Khalafallah NM. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. **Int. J. Pharm**. 2007;332(1-2):1-16.
69. Das SK, Chakraborty S, Roy C, et al. Ethosomes as novel vesicular carrier: An overview of the principle, preparation and its applications. **Curr. Drug Deliv.** 2018;15.
70. Robaire B, Henderson NA. Actions of 5α-reductase inhibitors on the epididymis. **Mol. Cell Endocrinol.** 2006;250(1-2):190-195.
71. Elsayed MA, Abdallah YO, Naggar FV, Khalafallah NM. Lipids vesicles for skin delivery of drugs: Reviewing three decades of research. **Int J Pharm**. 2006;332:1–16.
72. Raj Kumar Tiwari, Nitesh S Chauhan, Yogesh H S**,** Ethosomes: A Potential Carries For Transdermal Drug Delivery, International Journal of Drug Development & Research. 2010;2 (2):448 -452.
73. Rao LS. Preparation of liposomes on the industrial scale: Problems and perspectives. In: Gregoriadis G, editor. **Liposome Tech.**, Vol. 1. Florida: CRC Press; 1984.
74. New RRC. Preparation of liposomes. In: New RRC, editor. Liposomes-a practical approach. Oxford: Oxford University Press; 1990.
75. Mbah CC, Builders PF, Attama AA. Nanovesicular carriers as alternative drug delivery systems: ethosomes in

focus. **Expert Opin Drug Deliv.** 2014;11(1):45–59.

76. Elka Touitou., Dermal and transdermal drug delivery: Available at http://www.yissum.co.il/find-experts/expert/1185?rk=.
77. Sudhakar CK, Nitish U, Sanjay J, Charyulu RN. Ethosomes as non-invasive loom for transdermal drug delivery. **Nanomed. Drug Del.,** 2012:1–16.
78. Shen LN, Zhang YT, Wang Q, Xu L, Feng NP. Enhanced in vitro and in vivo skin deposition of apigenin delivered using ethosomes. **Int. J. Pharm**. 2014;460(1–2):280-288.
79. Park SN, Lee HJ, Gu HA. Enhanced skin delivery and characterization of rutin-loaded ethosomes. **Korean J. Chem. Eng**. 2014;31(3):485-489.
80. Ascenso A, Raposo S, Batista C, Cardoso P, Mendes T, Praca GF, et al. Development, characterization, and skin delivery studies of related ultradeformable vesicles: transfersomes, ethosomes, and transethosomes. **Int. J. Nanomed.**, 2015;10:5837-5851.
81. Paolino, D., Lucania, G., Mardente, D., Alhaique, F. and Fresta, M., 2005. Ethosomes for skin delivery of ammonium glycyrrhizinate: in vitro percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers. **J. Control. Rel.,** 2005;106(1-2):99-110.
82. Koli JR, Lin S. Development of anti oxidant ethosomes for topical delivery utilizing the synergistic properties of Vit A palmitate, Vit E and Vit C. **AAPS Pharm Sci Tec.** 2008;11:1–8.
83. Esposito E, Menegatti E, Cortesi R. Ethosomes and liposomes as topical vehicles for azeliac acid: A preformulation study. **J. Cosmet. Sci.** 2004;55:253–64.
84. Lodzki M, Godin B, Rakou L, Mechoulam R, Gallily R, Touitou E. Cannabiol-transdermal delivery and anti-inflammatory effect in a murine model. **J. Contr. Rel.,** 2003;93:377–87.
85. Touitou E, Godin B, Dayan N. Intracellular delivery mediated by ethosomal carrier. **Biomaterials.** 2001;22:3055–9.
86. Tunyaluk Limsuwan, Prapaporn Boonme, Pasarat Khongkow, Thanaporn Amnuaikit Ethosomes of Phenylethyl Resorcinol as Vesicular Delivery System for Skin Lightening Applications. BioMed Research International. 2017.
87. A. K. Barupal, Vandana Gupta and Suman Ramteke. Preparation and characterization of ethosomes for topical delivery of aceclofenac. Indian J Pharm Sci. 2010;72 (5): 582-586.
88. Yeh MI, Huang HC, Liaw JH, et al. Ethosomes in hair dye products as carriers of the major compounds of black tea extracts. **Int. J. Dermatol.** 2013;52(7):868–875.
89. Shen S, Liu SZ, Zhang YS, et al. Compound antimalarial ethosomal cataplasm: preparation, evaluation, and mechanism of penetration enhancement. **Int J Nanomed.**, 2015;10:4239–4253.
90. Liu X, Liu H, Liu J, et al. Preparation of a ligustrazine ethosome patch and its evaluation in vitro and in vivo. **Int. J. Nanomed.,** 2011;6:241–247.
91. Liu X, Liu H, Zeng Z, Zhou W, Liu J, He Z. Pharmacokinetics of ligustrazine ethosome patch in rats and anti-myocardial ischemia and anti-ischemic reperfusion injury effect. **Int J Nanomed.,** 2011;6:1391–1398.
92. Ahad A, Aqil M, Kohli K, Sultana Y, Mujeeb M. Enhanced transdermal delivery of an anti-hypertensive agent via nanoethosomes: statistical optimization, characterization and pharmacokinetic assessment. **Int. J. Pharm**. 2013;443(1–2):26–38.
93. Nagadevi B, Kumar KS, Venkanna P, Prabhakar D. Formulation and characterisation of tizanidine hydrochloride loaded ethosomes patch. **Int. J. Pharm. Pharm. Sci.** 2014;6(4):199–205.
94. Godin B, Touitou E. Ethosomes: new prospects in transdermal delivery. **Crit. Rev. Ther. Drug Carrier Syst.** 2003;20(1):63–102.
95. Kaur CD, Saraf S. Topical vesicular formulations of *Curcuma longa* extract on recuperating the ultraviolet radiation-damaged skin. **J. Cosmet. Dermatol.** 2011;10(4):260–265.
96. Gunjan J, Swarnlata S. Topical delivery of *Curcuma longa* extract loaded nanosized ethosomes to combat facial wrinkles. **J. Pharm. Drug Deliv. Res.** 3. 2014;3(1):1000118.
97. Horwitz E, Pisanty S, Czerninski R, Helser M, Eliav E, Touitou E. A clinical evaluation of a novel liposomal carrier for acyclovir in the topical treatment of recurrent herpes labialis. **Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.** 1999;87(6):700–705
98. Touitou E, Godin B, Shumilov M, et al. Efficacy and tolerability of clindamycin phosphate and salicylic acid gel in the treatment of mild to moderate acne vulgaris. **J. Eur. Acad. Dermatol. Venereol.** 2008;22(5):629–631.
99. Ainbinder D, Paolino D, Fresta M, Touitou E. Drug delivery applications with ethosomes. **J. Biomed. Nanotech.** 2010;6(5):558–568.
100. Hasanzadeh H, Nasrollahi SA, Halavati N, Saberi M, Firooz A. Efficacy and safety of 5% minoxidil topical foam in male pattern hair loss treatment and patient satisfaction. **Acta Dermatovenerol. Alp.** Pannonica Adriat. 2016;25(3):41-44.
101. Kanti V, Messenger A, Dobos G, et al. Evidence-based (S3) guideline for the treatment of androgenetic alopecia in women and in men – short version. **J. Eur. Acad. Dermatol. Venereol.,** 2018;32(1):11-22.
102. Blume-Peytavi U, Issiakhem Z, Gautier S, et al. Efficacy and safety of a new 5% minoxidil formulation in male androgenetic alopecia: A randomized, placebo-controlled, double-blind, noninferiority study. **J. Cosmet. Dermatol.** April 2018. (in press)
103. Nichols AJ, Hughes OB, Canazza A, Zaiac MN. An Open-Label Evaluator Blinded Study of the Efficacy and Safety of a New Nutritional Supplement in Androgenetic Alopecia: A Pilot Study. **J. Clin. Aesthet. Dermatol.** 2017;10(2):52-56.
104. Laïb S, Routh AF. Fabrication of colloidosomes at low temperature for the encapsulation of thermally sensitive compounds. **J. Colloid. Interface. Sci.** 2008;317(1):121-129.