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Ethosomes: A Novel Tool for Transdermal Drug Delivery

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ABSTRACT:

Transdermal drug delivery system was first introduced more than 20 years ago. Transdermal drug delivery system is a type of convenient drug delivery system where drug goes to the systemic circulation through the protective barrier i.e. Skin. Skin acts as a major target as well as a principal barrier for topical / transdermal drug delivery. Despite the many advantages of this system, the major obstacle is the low diffusion rate of drugs across the stratum corneum. One simple and convenient approach is application of drugs in formulation with elastic vesicles or skin enhancers. Vesicular system is one of the most controversial methods for transdermal delivery of active substances in that ethosome are the ethanolic phospholipids vesicles which are used mainly for transdermal delivery of drugs. Ethosomal drug delivery system is a new state of the art technique and easier to prepare in addition to safety and efficacy. Ethosomes have become an area of research interest, because of its enhanced skin permeation, improved drug delivery, increased drug entrapment efficiency etc. The purpose of writing this review on ethosomes drug delivery was to compile the focus on the various aspects of ethosomes including their mechanism of penetration, preparation, advantages, characterization, composition, application and marketed product of ethosomes. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies.

KEYWORDS: Ethosomes, Transdermal drug delivery, ethanol, phospholipid.

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TABLE CONTENTS:

1. INTRODUCTION:

FDA approved the first transdermal patch products in 1981. These delivery systems provided the controlled systemic absorption of scopolamine for the prevention of motion sickness (*Transderm-Scop*, ALZA Corp.) and nitroglycerine for the prevention of angina pectoris associated with coronary artery disease (Transderm-Nitro). Over the last two decades, more than 35 transdermal products have been approved generating sales of $3.2 billion in 2002, which is predicted to rise to $4.5 billion in 2008. More recently, such dosage forms have been developed and/or modified in order to enhance the driving force of drug diffusion (thermodynamic activity) and/or increase the permeability of the skin. These approaches include the use of penetration enhancers, supersaturated systems, prodrugs, liposomes and other vesicles.

One of the major advances in vesicle research was the finding that some modified vesicles possessed properties that allowed them to successfully deliver drugs in deeper layers of skin. Transdermal delivery is important because it is a noninvasive procedure for drug delivery. Further, problem of drug degradation by digestive enzymes after oral administration and discomfort associated with parenteral drug administration can be avoided. It is the most preferred route for systemic delivery of drugs to pediatric, geriatric and patients having dysphasia.

Despite the promise, there were many problems that researchers had to face with while attempting successful transdermal drug delivery. The skin is a multi-layered structure made up of stratum corneum (SC), the outermost layer, under which lies the epidermis and dermis. Within these layers of skin are interspersed fibroblasts, hair follicles and sweat glands that originate in the dermis blood supply. The almost insurmountable nature of SC is a major challenge for systemic delivery of percutaneously applied drugs [1]. The Òbrick and mortarÓ arrangement of corneocytes, flattened mononucleated keratinocytes, with interspersed lipids and proteins makes the SC approximately 1000 times less permeable than other biological membranes. Furthermore, it is even more difficult for anything to penetrate to the deeper strata of skin [2, 3].

To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier.

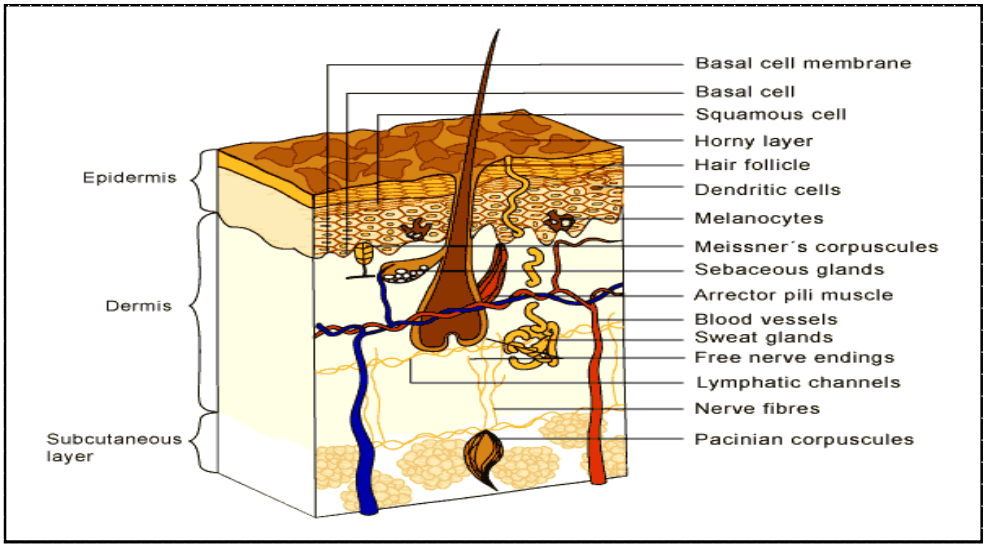

Figure 1: Structure of skin

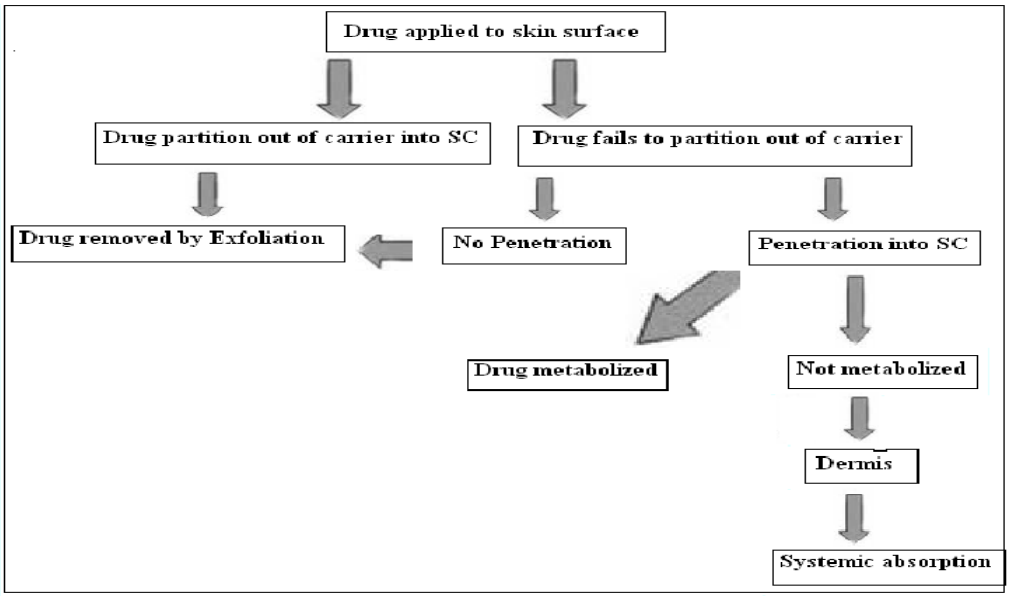

Figure 2: Proposed mechanism of drug absorption through skin

The non-invasive approaches for providing transdermal drug delivery of various therapeutic substances are:

Drug and vehicle interactions

- Selection of correct drug or prodrug
- Chemical potential adjustment
- Ion pairs and complex coacervates
- Eutectic systems

Stratum corneum modification

- Hydration

- Chemical penetration enhancers

Stratum corneum bypassed or removed

- Microneedle array
- Stratum corneum ablated
- Follicular delivery

Electrically assisted methods

- Ultrasound (Phonophoresis, Sonophoresis)
- Iontophoresis
- Electroporation
- Magnetophoresis
- Photomechanical wave

Vesicles and particles

- Liposomes and other vesicles
- Niosomes
- Transfersomes
- Ethosomes

2. Rational for Transdermal Drug Delivery:

Given that the skin offers such an excellent barrier to molecular transport, the rationale for this delivery strategy needs to be carefully identified. There are several instances where the most convenient drug intake methods the oral route) were not feasible and alternative routes had to be sought. Although, intravenous introduction of the medicament avoids many of these shortfalls (such as gastrointestinal and hepatic metabolism), its invasive and apprehensive nature (particularly for chronic administration) has encouraged the search for alternative strategies. Transdermal drug delivery (TDD) offers several distinct advantages including relatively large and readily accessible surface area (1Ð2 m^2) for absorption, ease of application and termination of therapy. Further, evolution of better technologies for delivering drug molecules, safe penetration enhancers and the use of vesicular carriers have rejuvenated the interest for designing TDD system for drugs that were thought to be unfit for transdermal delivery [4, 5].

3. Vesicular approaches for topical drug delivery:

Drug encapsulated in lipid vesicles prepared from phospholipids and nonionic surfactants is known to-be transported into and across the skin. Lipids present in the skin contribute to the barrier properties of skin and prevent systemic absorption of drugs. Due to the amphiphilic nature, lipid vesicles may serve

as non-toxic penetration enhancer for drugs. In addition, vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. Therefore, these lipid rich vesicles are hypothesized to carry significant quantity of drugs across the skin thus, enhancing the systemic absorption of drugs.

Drug delivery from liposomes in transdermal formulation has been studied for many purposes but unstable nature and poor skin permeation limits their use for topical delivery [6, 7]. In order to increase the stability of liposomes, the concept of proliposomes was proposed [8]. This approach was extended to niosomes, which exhibited superior stability as compared to liposomes [9]. However, due to poor skin permeability, liposomes and niosomes could not be successfully used for systemic drug delivery and their use was limited for topical use [10]. To overcome problems of poor skin permeability Cevc *et al.* [11] and Touitou *et al.* [12] recently introduced two new vesicular carrier systems transfersomes and ethosomes, respectively for non-invasive delivery of drugs into or across the skin. Transfersomes¨ and ethosomes incorporated edge activators (surfactants) and penetration enhancers (alcohols and polyols), respectively, to influence the properties of vesicles and stratum corneum [13]. The vesicles have been well known for their importance in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicles structure for use in better drug delivery within their cavities, which would to tag the vesicle for cell specificity. One of the major advances in vesicle research was the finding a vesicle derivatives, known as an Ethosomes [14].

4. Ethosomes:

Ethosomes are the 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. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers (nm) to microns ( ) ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux [15, 16].

4.1 Structure of Ethosomes:

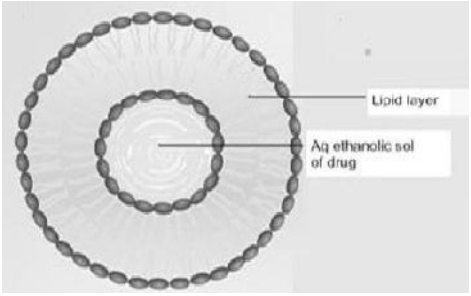

Figure 3: Structure of Ethosomes

4.2 Ethosomes Composition:

The ethosomal system consists of phospholipids, ethanol and water [17]. The phospholipids with various chemical structure includes phosphatidylcoholine (PC), hydrogenated PC, phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PPG), phosphatidy linositol (PI), hydrogenated PC etc [18]. The nonaqueous phase range between 22 % to 70 %. The alcohol may be ethanol or isopropyl alcohol. Dyes or amphiphilic fluorescent probe such as D – 289, Rhodamine – 123 , fluorescence isothiocynate (FITC), 6 – carboxy fluorescence are often added to ethosomes for characterization study [19, 20].

Table 1: Different Additives Employed In Formulation of Ethosomes

Class	Example	Uses
Phospholipid	Soya phosphatidyl choline Egg phosphatidyl choline Dipalmityl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane As a penetration enhancer
Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123 Rhodamine red Fluorescene Isothiocynate (FITC) 6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol Đ934	As a gel former

Effect of high alcohol concentration:

Ethanol is an established permeation enhancer and is proposed that it fluidizes the ethosomal lipids and stratum corneum bilayer thus allowing the soft, malleable vesicles to penetrate the disorganized lipid bilayer [21]. The relatively high concentration of ethanol (20 – 50 %) is the main reason for better skin permeation ability and is packed less tightly than conventional vesicles but has equivalent stability and better solubility of many drugs [19,22]. Moreover the vesicular nature of ethosomal formulation could be modified by varying the components ratio and phospholipids [23]. Ethanol confers a surface negative net charge to the ethosome which causes the size of vesicles to decrease. The size of ethosomal vesicles increase with decreasing ethanol concentration [19].

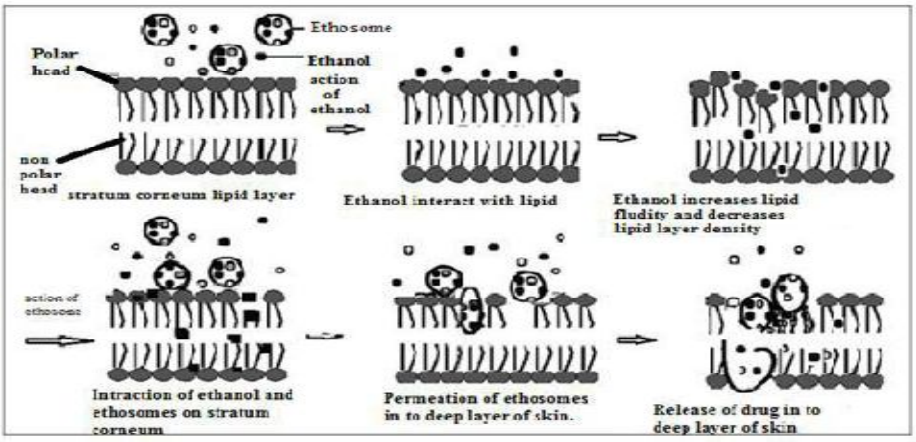

Figure 4: Proposed mechanism for penetration of molecule from ethosomal system across the lipid domain of stratum corneum

The enhanced delivery of actives using ethosomes over liposomes can be ascribed to an interaction between ethosomes and skin lipids. A possible mechanism for this interaction has been proposed. It is thought that the first part of the mechanism is due to the 'ethanol effect whereby intercalation of the ethanol into intercellular lipids increasing lipid fluidity and decreases the density of the lipid multilayer.

This is followed by the 'ethosome effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin as shown in Figure 4. The drug absorption probably occurs in following two phases:

Ethanol effect: Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

Ethosomes effect: Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin permeability. So the ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.

4.3 Advantages of ethosomes:

1.Enhanced permeation of drug molecules to and through the skin to the systemic circulation [19, 24].

2. Contrary to deformation liposomes, ethosomes improve skin delivery of drugs both under occlusive and non-occlusive conditions [25].

3. Since composition and components of ethosomes are safe, they have various applications in pharmaceutical, veterinary and cosmetic field.

4. Better patient compliance.

5. Better stability and solubility of many drugs as compared to conventional vesicles.

7. Relatively smaller size as compared to conventional vesicles.

4.4 Limitations of ethosomes:

1. Poor yield [26].

2. In case if shell locking is ineffective then the ethosomes may coalescence and fall apart on transfer into water.

3. Loss of product during transfer form organic to water media [27].

4.5 Methods of preparation ethosomes:

Ethosomes can be prepared by two very simple and convenient methods that is hot method (Fig 7) and cold method (Fig.6) [19, 20, 21, 28].

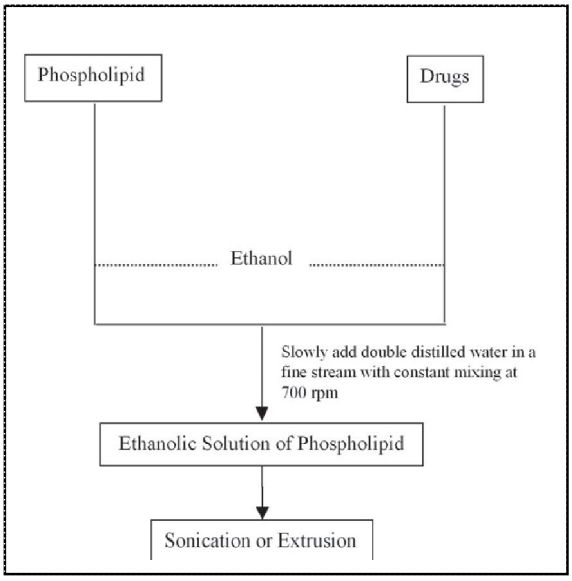

Figure 5: General method for the preparation of ethosomes

A. Cold Method:

This is the most common method utilized 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 mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 30^0C in a water bath. The water heated to 30^0C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of

ethosomal formulation can be decreased to desire extend using sonication [29] or extrusion [30] method. Finally, the formulation is stored under refrigeration[31].

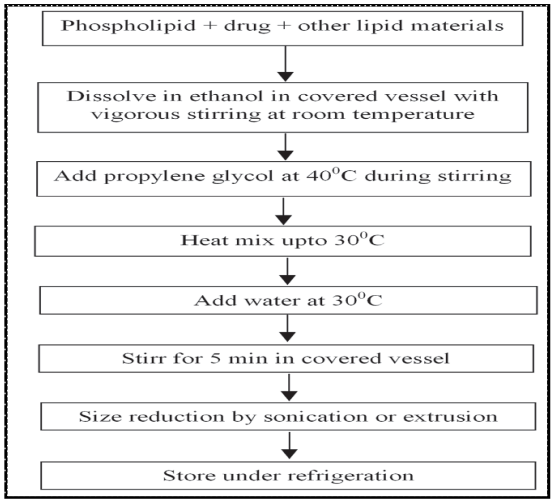

Figure 6: Cold method for the preparation of ethosomes.

B. Hot method:

In this method phospholipid is dispersed in water by heating in a water bath at 40^{0}C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40^{0}C. Once both mixtures reach 40^{0}C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties[32, 31]. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.

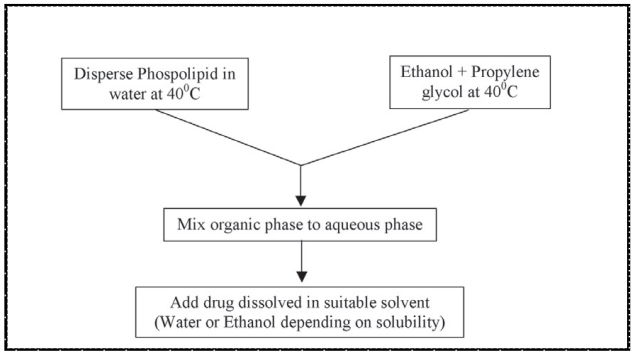

Figure 7: Hot method for the preparation of ethosomes

4.6 Characterizations of Ethosomes

4. 6.1. Visualization [33]

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

4.6.2. Vesicle size and Zeta potential [34]

Particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS).

4.6.3. Entrapment Efficiency [35]

The entrapment efficiency of drug by ethosomes can be measured by the ultra centrifugation technique.

4.6.4. Transition Temperature [36]

The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry.

4.6.5. Surface Tension Activity Measurement [37]

The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

4.6.6. Vesicle Stability [38]

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

4.6.7. Penetration and Permeation Studies [39]

Depth of penetration from ethosomes can be visualized by confocal laser scanning.

4.7 Evaluation Tests:

4.7.1. Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy [40]

Vesicle suspension (0.2 mL) was applied to filter membrane having a pore size of 50 nm and placed in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with PBS (phosphate buffer saline solution), (pH 6.5). The filters were removed after 1 hour and prepared for SEM studies by fixation at 4°C in Karnovsky's fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% vol/vol in water). Finally, filters were coated with gold and examined in SEM (Leica, Bensheim, Germany).

4.7.2. Skin Permeation Studies

The hair of test animals (rats) were carefully trimmed short (<2 mm) with a pair of scissors, and the abdominal skin was separated from the underlying connective tissue with a scalpel. The excised skin was placed on aluminium foil, and the dermal side of the skin was gently teased off for any adhering fat and/or subcutaneous tissue. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm2 and 10 mL, respectively. The temperature was maintained at 32°C ± 1°C. The receptor compartment contained PBS (10 mL of pH 6.5). Excised skin was mounted between the donor and the receptor compartment. Ethosomal formulation (1.0 mL) was applied to the epidermal surface of skin. Samples (0.5 mL) were withdrawn through the sampling port of the diffusion cell at 1-, 2-, 4-, 8-, 12-, 16-, 20-, and 24-hour time intervals and analyzed by high performance liquid chromatography (HPLC) assay.

4.7.3. Stability Study

Stability of the vesicles was determined by storing the vesicles at 4°C ± 0.5°C. Vesicle size, zeta potential, and entrapment efficiency of the vesicles was measured after 180 days using the method described earlier.

4.7.4. Vesicle-Skin Interaction Study by TEM and SEM

From animals ultra thin sections were cut (Ultracut, Vienna, Austria), collected on formvar-coated grids and examined under transmission electron microscope. For SEM analysis, the sections of skin after dehydration were mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater. The sections were examined under scanning electron microscope.

4.7.5. Vesicle-Skin Interaction Study by Fluorescence Microscopy

Fluorescence microscopy was carried according to the protocol used for TEM and SEM study. Paraffin blocks are used, were made, 5- m thick sections were cut using microtome (Erma optical works, Tokyo, Japan) and examined under a fluorescence micro Cytotoxicity Assay MT-2 cells (T-lymphoid cell lines) were propagated in Dulbecco's modified Eagle medium (HIMEDIA, Mumbai, India) containing 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L L-glutamine at 37°C under a 5% CO2 atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at 540 nm.

4.7.6. Drug Uptake Studies

The uptake of drug into MT-2 cells (1×106 cells/mL) was performed in 24-well plates (Corning Inc) in which 100 L RPMI medium was added. Cells were incubated with 100 L of the drug solution in PBS (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay.

4.7.7. HPLC Assay

The amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cell was determined by HPLC assay using methanol: distilled-water :acetonitrile (70:20:10 vol/vol) mixture as mobile phase delivered at 1 mL/min by LC 10-AT vp pump (Shimadzu, Kyoto, Japan). A twenty-microliter injection was eluted in C-18 column (4.6×150 mm, Luna, 54, Shimadzu) at room temperature. The column eluent was monitored at 271 nm using SPD-M10A vp diode array UV detector. The coefficient of variance (CV) for standard curve ranged from 1.0% to 2.3%, and the squared correlation coefficient was 0.9968.

4.7.8. Statistical Analysis

Statistical significance of all the data generated was tested by employing ANOVA followed by studentized range test. A confidence limit of $P < .05$ was fixed for interpretation of the results using the software PRISM (GraphPad, Version 2.01, San Diego, CA).

4.8 Different Studies Related to the Application of Ethosomes as a Carrier System:

Various studies employing ethosomal formulation have shown better skin permeability of drugs. The uses of ethosomes as carrier system for transdermal/topical drug delivery are summarized below.

4.8.1. Pilosebaceous targeting: Pilosebaceous units have been use for localized therapy, particularly for the treatment of follicle related disorders such as acne or alopecia. Ethosomal formulation of minoxidil a lipid soluble drug used for baldness accumulate into nude mice skin two to seven fold higher and thus can be use for pilosebaceous targeting for better clinical efficacy [51,60].

4.8.2. Transdermal delivery: Since ethosomes enhance permeability of drug through stratum corneum barrier, it can be use for administration of drugs having poor skin permeation, low oral bioavability, first pass metabolism and dose skin and suppress infection at their root [60, 48, 61].

Table 2: Application of Ethosomes as a Drug Carrier

Drugs	Results
Anti- viral agents (Zidovudine) [41] (Lamivudine)[42] (Stavudine) [43]	Prolonged drug action, reduced drug toxicity. Control release for prolonged period of time. Improved biological anti-inflammatory activity, sustained effect
NSAIDS [44,45] (Diclofenac) (Aceclofenac)	Selective and prolong delivery of drug to desired site. Superior to the marketed gel for the topical administration.
Acyclovir [46]	Increased skin permeation and biological activity two to three times.
Topical [47] Photodynamic Therapy (PDT) (5- aminolevulinic acid)	Greater penetration ability than that of liposomes, More entrapment efficiency
Insulin [48, 49]	Significant decrease in blood glucose level.
Trihexyphenidyl Hydrochloride[49]	Higher entrapment capacity, improved tansdermal flux, improved) patient compliance.
Antibiotic [50] (Erythromycin) (Cannabidol)	Complete inhibition of infection, prolonged drug action. Improved skin deposition and biological activity.
Pilosebaceous [51] (Minoxidil)	High penetration into deep layers of the skin. Targeting
Ammonium [52] Glycrrhizinate	Improved biological anti-inflammatory activity, sustained effect.
Salbutamol sulfate [53]	Controlled release rate, enhanced skin permeation.
Propranolol [54]	Better skin permeation.
Testosterone [55]	Significantly higher permeation into the skin increased systemically delivery
Finasteride [56]	Enhanced percutaneous absorption.
Bacitracin [57]	Reduced drug toxicity.
Methotrexate [58] (MTX)	Enhanced transdermal flux, lower lag time, higher entrapment efficiency and better stability profile
Gold Nanopartical [59]	Gold nanopartical in ethosomes shows enhancement of pharmacological efficacy in transdermal and dermal delivery systems.

4.8.3. Delivery of HIV drugs: An effective antiretroviral therapy is required on a long term basis and is associated with strong side effects [62]. Adequate zero order delivery of zidovudine, Lamivudine a potent antiviral agent is required to maintain expected anti – AIDS effect. Subheet Jain et al reported that ethosomal formulation of the above drugs prolong the release with increased transdermal flux [52].

Conventional topical preparation acyclovir an topically used antiviral drug for treatment of herpes labials show low therapeutic efficiency due to poor permeation through skin as replication of virus take places at the basal dermis. Ethosomal formulation of acyclovir show high therapeutic efficiency with shorter healing time and higher percentage of abortive lesions.

4.8.4. Delivery of problematic drug molecules:

Oral delivery of large biogenic molecules such as peptides or proteins and insulin is difficult because they are completely degraded in the GIT tract hence transdermal delivery is a better alternative. But conventional transdermal formulation of biogenic molecules such as peptides or protein and insulin has poor permeation. Formulating these above molecules into ethosomes significantly increase permeation and therapeutic efficacy [63].

5. Patented and marketed formulation of ethosome:

Ethosome was invented and patented by Prof. Elka Touitou along with her students of department of Pharmaceutics at the Hebrew University School of Pharmacy [44, 45]. Novel Therapeutic Technologies Inc (NTT) of Hebrew University have been succeeded in bringing a number of products to the market based on ethosome delivery system. Noicellex TM an anti – cellulite formulation of ethosome is currently marketed in Japan. Lipoduction TM another formulation is currently used in treatment of cellulite containing pure grape seed extracts (antioxidant) is marketed in USA. Similarly Physonics is marketing anti – cellulite gel Skin Genuity in London. Nanominox© containing monoxidil is used as hair tonic to promote hair growth is marketed by Sinere.

6. Future Prospects

Introduction of ethosomes has initiated a new area in vesicular research for transdermal drug delivery. Different reports show a promising future of ethosomes in making transdermal delivery of various agents more effective. Further, research in this area will allow better control over drug release in vivo, allowing physician to make the therapy more effective. Ethosomes offers a good opportunity for the non-invasive delivery of small, medium and large sized drug molecules. The results of the first clinical study of acyclovir-ethosomal formulation support this conclusion. Multiliter quantities of ethosomal formulation can be prepared very easily. It, therefore, should be not before long that the corresponding drug formulation would have found their way into clinics to be tested for widespread usage. Thus, it can be a logical conclusion that ethosomal formulations possess promising future in effective dermal/transdermal delivery of bioactive agents.

REFERENCES:

1. Barry B W. Novel mechanisms and devices to enable successful transdermal drug delivery, Eur.J.Pharm. Sci. 2001;14 :101-114.
2. Hadgraft J S. The final frontier, Int. J. Pharm. 2001;14: 224, 1-18.
3. Hadgraft J S. Structure and Function of the Stratum corneum as Border Organ, Skin Pharmacology and Applied Skin Physiology 2001;14 : 72-81.
4. Guy R H. Ethosomes an recent approach in transdermal drug delivery system. Int.J. Pharm. 1985;6 : 112-116.
5. Panchagnula R, Pillai O, Nair V.B, Ramarao P. Transdermal iontophoresis revisisted. Current Opinion in Chem. Bio. 2000;4 : 468-473.
6. Mezei M, Gulusekharam V. Liposomes - a selective drug delivery system for the topical route of administration. Life Science. 1980;26 : 1473-1477.
7. Yarosh D B. Liposomes in investigative dermatology. Photoimmunology and Photomed. 2001;17 : 203-212.
8. Deo M R, Sant V P, Prakash S R, Khopade A J, Banakar U V. Proliposomes based transdermal delivery of levonorgestrel. J. Bio.Appl. 1997; 12: 77-85.
9. Vora B, Khopade A J, Jain N K. Proniosome based transdermal delivery of levonorgestrel for effective contraception. J. Cont. Release. 1998; 54: 149-165.
10. Lasch J, Laub P, Wohlrab W. How deep do intact liposomes penetrate to human skin. J. Cont. release 1991; 18 : 55-58.
11. Cevc G, Blume G, Schatzlein A, Gebauer D, Paul A. The skin pathway for systemic treatment patches and lipid based agent carriers. Advanced Drug Delivery Reviews. 1996;18 : 349-378.
12. Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes - novel vesicular carriers for enhanced delivery: characterization and skin penetration properties .J.Cont. Release ; 2000;65 : 403-418.
13. Cevc G, Blume G, Schatzlein A. Transfersomes-mediated transepidermal delivery improves the regio-specificity and biological activity of corticosteroids in vivo. J.Cont. Release 1997;45; 211-226.
14. Jain N K, Advances in controlled and novel drug delivery. 1st edition. New Delhi. CBS Publication. 2001; 428-451.
15. Touitou E I, Composition of applying active substance to or through the skin. US patent 5 540 934, 1996, July 30.

16. Toutitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. Journal of Controlled Release 2000; 65 :403 – 418.
17. Touitou E. Drug delivery across skin. Expert Opinion on Biological Therapy 2002; 2: 723 – 733.
18. Schreier H, Bovwstra J. Liposomes and niosomes as topical drug carriers: dermal and transdermal drug delivery. Journal of Control Release 1994; 30: 1 – 15.
19. Touitou E. Composition of applying active substance to or through the skin. US Patent:5716638, 1996.
20. Touitou E, Composition of applying active substance to or through the skin. US Patent: 5540934, 1998.
21. Dinesh D, Amit A R, Maria S, Awaroop R L, Mohd Hassan G D. Drug vehicle based approaches of penetration enhancement. Int. J. Pharm. Pharm. Sci. 2009; 1(1) : 24 – 45.
22. Barry B W. Is Transdermal drug delivery research still important today. Drug Discovery Today 2001;6(19) : 967 – 971.
23. Biju S S, Sushama T, Mishra P R, Khar R K. Vesicular systems: An overview. Ind.J. Pharma. Sci. 2006; 68(2) : 141-153.
24. Dayan N, Touitou E. Carriers for skin delivery of triexphenidyl HCl: Ethosome Vs Lipsomes. Biomaterials 2000;21 : 1879 – 1885.
25. Elsayed M M, Abdallah O Y, Naggar V F, Khalafallah N M. Deformable liposomes and ethosomes as carriers for skin delivery of ketotifen. Pharmazie. 2007;62 : 133 – 137.
26. Laib S, Routh A F. Fabrication of colloidosomes at low temperature for the encapsulation of thermally sensitive compounds. J. Colloid & Interface Sci. 2008;317 : 121-129.
27. Swarnlata S, Rahul R, Chanchal D.K, Shailendra S. Colloidosomes an advanced vesicular system in drug delivery. Asian J.Sci. Research 2011;4(1) : 1 – 15.
28. Verma D D, Fahr A. Synergistic penetration effect of ethanol and phospholipids on the topical delivery of cyclosporine. J.Cont. Release. 2007;97(1) : 55 – 66.
29. Jain S, Umamaheshwari R.B, Bhadra D, Jain N.K, Ethosomes: a novel vesicular carrier for enhanced transdermal delivery of an anti-HIV agent. Ind. J. Pharm. Sci. 2004;66 : 72-81.
30. Verma D D, Fahr A. Synergistic penetration effect of ethanol and phospholipids on the topical delivery of Cyclosporin. J. Cont.Release 2004;97: 55-66.

31. Touitou E. Composition of applying active substance to or through the skin., US patent, 5,540,934, 1998**.**
32. Touitou E. Composition of applying active substance to or through the skin., US patent, 5,716,638, 1996.
33. Touitou E. Compositions for applying active substances to or through the skin. US Patent 5 538, 934, 19.
34. Maghraby G.M, Williams A.C, Barry B.W. Oestradiol skin delivery from ultra deformable liposomes: refinement of surfactant concentration. Int. J. Pharma. 2000: 63-74.
35. Fry D W, White J.C, Goldman I.D. Rapid secretion of low molecular weight solutes 1from liposomes without dilution. Analytical Biochemistry 1978;90: 809- 815.
36. New R C. Preparation of liposomes and size determination, In:Liposomes A Practical Approach, New RRC (Ed.), Oxford University Press, Oxford. 1990; 36-3.
37. Cevc G, Schatzlein A, Blume G. Transdermal drug carriers: Basic properties, optimization and transfer efficiency in case of epicutaneously applied peptides. J. Cont. Release 1995;36: 3-16.
38. Berge V, Swartzendruber V.B, Geest J. Development of an optimal protocol for the ultrastructural examination of skin by transmission electron microscopy. J. Microscopy 1997; 187(2): 125-133.
39. Toll R, Jacobi U, Richter H, Lademann J, Schaefer H, Blume U. Penetration profile of microspheres in follicular targeting of terminal hair follicles. J. Investigative Dermatology 2004; 123: 168-176.
40. Lopez-Pinto J.M, Gonzalez-Rodriguez M.L, Rabasco A.M. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. Int. J. Pharma. 2005;298 :1-12.
41. Jain S, Umamaheshwari R.B, Bhadra D, Jain N.K. Ethosomes – A novel vesicular carrier for enhanced Transdermal delivery of an anti –HIV agent. Ind.J.Pharm. Sci.2004;66(1): 72– 81.
42. Subjeet J, Ashok K.T, Bharti S, Narendra K.J. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. AAPS Pharm SciTech 2007; 8(4): E1 – E9.
43. Sheo D.M, Sunil K.P, Anish K.G, Gyanendra K.S, Ram C.D. Formulation development and evaluation of ethosome of stavudine. Ind. J. Pharm. Edu. Res. 2010; 44(1): 102 – 108.
44. Touitou E. Composition of applying active substance to or through the skin. US Patent: 5716638, 1996.
45. Touitou E. Composition of applying active substance to or through the skin. US Patent: 5540934, 1998.

46. Barry B.W. Novel mechanism and devices to enable successful Transdermal drug delivery. Eur. J. Pharma. Sci. 2001; 14(2): 101 – 114.
47. Yi-Ping F, Yi-Hung T, Pao-chu W,Yaw – Bin H. Comparison of 5 aminolevulinic acid – encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. Int. J. Pharma. 2008;356: 144 – 152.
48. Banga A.K, Chein Y.W. Hydrogel – based iontotheraputic delivery devices for transdermal delivery of peptide/ protein drugs. Pharmaceutical Research 1993;10: 697 – 702.
49. Dayan N, Touitou E, Carriers for skin delivery of triexphenidyl HCl Ethosome Vs Lipsomes. Biomaterials 2000;21: 1879 – 1885.
50. Godin B, Touitou E, Rubinstein F, Athamna A, Athamna M, A new approach for treatment of deep skin infections by an ethosomal antibiotic preparation: an in vivo study. Journal of Antimicrobial Chemotherapy 2005;55(6): 989 – 994.
51. Toutitou E, Dayan N, Bergelson L, Godin B and Eliaz M. Ethosomes novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. J. Cont. Release 2000;65: 403 – 418.
52. Donatella P, Giuseppe L, Domenico M, Franco A, and Massimo F. Ethosomes for skin delivery of ammonium glycyrrhizinate permeation through human skin and in vivo anti inflammatory activity on human volunteers. J.Cont. Release 2005; 106: 99 – 110.
53. Ehab R.B, Mina I.T. Enhanced transdermal delivery of salbutamol sulfate via ethosomes. AAPS Pharm SciTech 2007; 8(4): E1 – E8.
54. Kirjavainen M, Urtti A, Valjakka – Koskela R, Kiesvaara J. Monkkoonen J. Liposome – skin interactions and their effects on the skin permeation of drugs. Eur. J. Pharm. Sci. 1999;7(4): 279 - 286.
55. Kaplun – Friscкhoff Y, Touitou E. Testosterone skin permeation enhancement by menthol through formation of eutectic with drug and interaction with skin lipids. J. Pharma. Sci. 1997;86: 1394 – 1399.
56. Yuefeng R, Feiyue Z, Xingguo Z, Jianqing G, Wenquan L. *In vitro* percutaneous permeation and skin accumulation of finasteride using vesicular ethosomal carriers. AAPS Pharm. Sci. Tech , 2008;9 (3): 860 – 865.
57. Godin B, Touitou E. Mechanism of bacitracin permeation enhancement through the skin and cellular membrane from an ethosomal carrier. J. Cont.Release 2004; 94: 365 – 379.

58. Vaibhav D, Dinesh M, Tathagata D *et al* Dermal and transdermal delivery of an anti – psoriatic agent via ethanolic liposomes. J. Cont. Release 2007; 123: 148 – 154.
59. Patricia de la, P, *et al.* Gold nanoparticals generated in ethosomes bilayers, as revealed by cryo –electron – tomography. J. Phy. Chem. 2009;113(10): 3051 – 3057
60. Biju S S, Sushama T, Mishra P R, Khar R K. Vesicular systems: An overview. Ind. J. Pharma. Sci. 2006;68 (2): 141-153.
61. Jia-You F, Chi-Tzong H, Wen-Ta C, Ying-Yue W. Effect of liposomes and niosomes on skin permeation of enoxacin. Int. J. Pharma. 2001; 219 (1): 61 – 72.
62. Jarivis B, Faulds D. Lamivudine: a review of its therapeutic potential in chronic hepatits B Drugs 1999; 58(1): 101 – 141.
63. Jain S, Jain P, Jain N.K. Transfersomes: a novel vesicular carrier for enhanced transdermal delivery: development, characterization and performance evaluation. Drug Development and Industrial Pharmacy 2003; 29: 1013 – 1026.