ETHOSOMES: A NOVEL NANO VESICULAR CARRIER FOR ENHANCED DERMAL, TRANSDERMAL AND INTRACELLULAR DRUG DELIVERY

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Abstract

Ethosomes are ethanolic liposomes, a novel lipid vesicular carrier prepared using phospholipid, ethanol and water. Instead of rupturing the liposomal structure while increasing the ethanol concentration, vesicles superior in delivering the active ingredient into the deeper layers of the skin was obtained, named as ethosomes. In contrast to the liposomes and deformable liposomes, ethosomes have been shown to exhibit high encapsulation efficiency, flexibility, higher *invitro* and *invivo* skin permeability and skin deposition ability for a wide range of molecules including lipophilic drugs and are effective to deliver the drug molecules to and through the skin. Intracellular delivery of ethosomal component was also evident, when labeled phospholipids used in the preparation of ethosomal vesicles. These studies clarified that ethosomal suspensions are not only effective in the transdermal delivery but also effective against intracellular infections, gene delivery, vaccination and cellular transformations in the biomedical research. The main aim of this article is to provide an overview of the ethosomes about its mechanism of enhanced permeation, dermal, transdermal and intracellular delivery of drugs with adequate discussions on recent advances in this field.

Key words: ethosomes, ethanolic liposomes, skin targetting, transdermal, vesicular drug delivery, liposomes

1. INTRODUCTION

Skin is an excellent organ for drug delivery having numerous advantages, starting from its surface area. Skin covers the total surface area of 1.8 m^2 approximately. Everyone can think that a huge amount of drug may be administered as transdermal delivery by using this large surface area. But the acceptable size of the transdermal patches restricts the maximum dose administered which is 10mg per day [1]. Skin also offers the advantages like targeting of drugs to the local infections or skin disorders, bypassing from hepatic first pass metabolism, discontinuation of the administration by removing the patches whenever needed, lowering of fluctuations in plasma drug concentrations, sustained as well as controlled administration of the required dose of drug etc. Even though, the skin having many advantages, the transdermal administration of drug molecules still faces some problems due to its barrier nature. The answer for the question "why barrier nature?" is in anatomical structure of the skin, that is stratum corneum. The actual major function of the skin is to protect the body from the invasion of exogenous substances, microbes and to prevent the loss of endogenous substances. Stratum corneum is doing its role wonderfully in its protective function, at the same time provides the obstacle for the permeation of drug molecules through the skin. The stratum corneum is the outermost layer of the skin, composed of keratin filled, solidified lamellar lipid surrounded dead cells called corneocytes. The densely cross linked nature of the protein keratin, crystalline long chain lipids having the lengthy acyl chains and overall tightly bounded nature of the stratum corneum attribute toward its barrier nature. In the past three decades of research, many techniques were discovered and applied to overcome this problem, which starts from the use of permeation enhancers in the transdermal patches followed by the discovery of micelles, vesicular carriers like liposomes, transferosomes, niosomes, flexosomes, pharmacosomes, vesosomes, invasomes, lipid nanocarriers like solid lipid nanoparticles(SLN), nanostructured lipid carriers, nano emulsions and polymeric nanoparticles. The inclusion of chemical penetration enhancers in transdermal delivery having high affinity with the skin lipids alters the physiochemical nature of stratum corneum and thereby enhances the drug permeation. But the use of surfactants and some other chemical enhancers induce irritation on the skin which

causes damage and hence alters the normal physiological nature of the skin. Therefore, adequate care should be taken by the scientists involved in the development of transdermal drug delivery systems and use of chemical enhancers which alter the normal barrier function of the skin should be avoided. The discovery of the novel drug carriers like micelles, vesicular carriers, nanoparticles was somewhat advantageous in certain aspects when compare to the chemical permeation enhancers in transdermal drug delivery systems. Instead of surfactants, alcohols like ethanol and propylene glycol in lesser concentration were also used as penetration enhancers in the liposomes. Usually alcohols were used in low concentrations (almost below 10%) in liposomal formulations analyzed for transdermal delivery of drugs. At that time scientists might think that the increase in alcohol concentration more than 10% will cause destruction of the vesicular structure or bilayer nature of the liposomes. Therefore, no one tried to use the alcohols beyond this limit. Toutou et al., increased the ethanol concentration from 10% up to 45% to prepare the vesicles which results in the invention of novel liposomes called ethosomes [2, 3]. The results also proved that the increasing concentration of ethanol does not destruct the vesicular structure, conversely produces the vesicles with higher entrapment efficiency, enhanced skin penetration and higher skin deposition. This novel vesicular carrier was analyzed to deliver a wide range of drug molecules including lipophilic drugs like minoxidil [4], cannabinoids [5] testosterone [6] and cationic drugs like propranolol, trihexyphenidyl etc [2]. In the recent past years, many research works were published on ethosomes containing acyclovir [7], fluorescent probes [8], bacitracin [9], azelaic acid [10], minoxidil [2, 11, 12], erythromycin [13], β-carotine [11], ammonium glycyrrhizinate [14], ketotifen [15], melatonin [16], lamivudine [17], solbutamol sulphate [18], methotrexate [19], vitamin E [20], temoporfin [21], 5-amino levulenic acid [22,23], fluconazole [24], matrine (a sophora flavescens alkaloid widely applied for inflammatory conditions) [25], miconazole [26], buspirone [27], diclofenac potassium [28], indinavir [29]. Ethosomes are superior over transdermal drug delivery systems in certain aspects like faster rate of drug delivery, delivery of wide range of molecules including peptides and highly lipophilic drugs, very simple preparation procedure and low cost of production, passive and noninvasive drug delivery, usefulness in pharmaceutical, veterinary and cosmeceuticals etc.

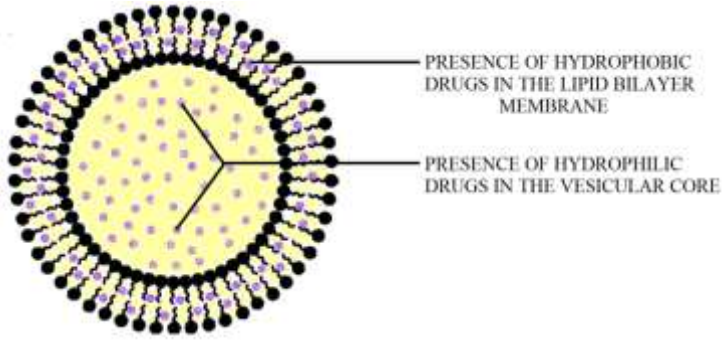

Fig. 1: An illustration explaining the structure of an ethosome vesicles and location of the hydrophilic and lipophilic drugs

E.Touitou et al proved the higher rate of drug delivery from ethosomes than the transdermal patches. An ethosomal formulation containing testosterone and a commercially available testosterone transdermal patches were compared for both in vitro and *invivo* drug permeation studies. Drug permeation from the ethosomal formulation showed 30 times higher release than from the transdermal patches in 24 hours. The skin deposition was also favored by the ethosomal formulation which is 7 times more than the transdermal patch. *Invivo* studies on rabbits revealed the superiority of ethosomes over transdermal patch which showed 125% more area under the curve (AUC) in ethosomes than the transdermal patches. It was also noted that none of the rabbits suffered from irritation after receiving ethosomal formulations which contains safe and approved components. Before discussing this interesting novel vesicular carrier in detail a brief discussion on some other novel carriers and its compositions used in transdermal delivery will add some interest.

2. NOVEL CARRIERS FOR DERMAL AND TRANSDERMAL DELIVERY

Liposomes are lipid vesicles made up of phospholipids; enclosing an aqueous volume [30]. Cholesterol may also be used as an additive to stabilize the vesicle structure by decreasing the permeability and increasing the rigidity of the vesicles [31]. In early stages

of discovery the liposomes approximately up to 1980, they were used as a carrier for oral and intravenous delivery of drugs, but not as topical or transdermal delivery of drugs. The topical application of liposomes was first introduced by Meizei and Gulasekharam in 1980 [32]. In the continuation of their research, a number of publications were made on the potential application of liposomes as dermal and transdermal carriers. But, it was discovered later that liposomes were not able to deliver efficiently the drug to the systemic circulation. Liposomes can improve the skin deposition of drug, thereby acts as a potential carrier for topical application, but not suitable for transdermal delivery. B.A.Van den Bergh et al and M.Kirijavanan et al., applied the liposomal formulation on the skin and investigate the interaction of liposomes with the skin [33-35]. They confirmed that the vesicular structures were confined only in the upper layers of the stratum corneum probably due to the desqamation of the corneocytes. Liposomes can also be used to target the skin appendages, sweat gland and sebaceous glands.

The discovery of micelles and liposomes are the basement for the introduction of many novel vesicular carriers. Liposomes are rigid in nature due to stabilization of bilayer by the use of cholesterol. Rigidity of vesicles improved by cholesterol, similarly fluidization or flexibility of the liposomes was improved by the addition of surfactants as edge activator. This discovery leads to a new class of highly deformable liposomes called transferosomes [36, 37]. Cevic and Blume et al., introduced the first transferosomes consisting of phospholipid to construct the bilayer and a single chain surfactant to stabilize the lipid bilayers which results the highly deformable vesicles [38, 39]. Sodium cholate, sodium deoxy cholate, tween 20, tween 60, tween 80, span 60, span 80 were used as edge activators and many publications were made on this novel vesicular carrier [40-44]. Lidocaine [45], cyclosporine [46], diclofenac [38], triamcinolone acetonide [37], zidovudine [47], hydrocartizone and dexamethazone [48], hormones like insulin [49], levonorgestrel [50], ethinyl estradiol [43] and low molecular weight heparin [51] were investigated for dermal and transdermal application *invivo* by this type of carrier. The immunization potential of transferosomes was reported using tetanus toxoid and confirmed the superiority in comparison with the intramuscular immunization. Oestradiol, cyclosporine A, 5-fluorouracil, methotrexate, diclofenac, melatonin, ketotifen etc were reported as transferosomes in *in vitro* studies for enhanced transdermal delivery of drugs. Y.K.Song and C.K.Kim prepared the liposomes containing biocompatible membrane softener and named as flexible liposomes also called flexosomes [51]. Flexible liposomes vesicles bearing anionic, cationic and neutral charges were prepared using dicetyl phosphate, 1,2 Diolaoyl-3-trimethyl ammonium propane and egg yolk L-α-phosphatidyl choline respectively. Flexosomes overcome the barrier nature of the skin by interacting with hydrophilic pathways through the skin.

Instead of encapsulation of drug in the core or bilyer membrane as in liposomes, transferosomes, niosomes etc, the drug bounds covalently with a lipid molecule and aggregate themselves to form vesicular, micellar hexagonal aggregation to form a new class of carrier called pharmacosomes [52-55]. The main advantages of this carrier over the other vesicular carrier includes prevention of leakage from vesicle due to the covalent bond between drug and lipid molecule, predetermined high entrapment efficiency due to pre estimation of amount of drug in conjugation with lipids, improved physiochemical stability depends upon the properties of drug lipid complex etc.

The presence of surfactants in the lipid bilayer increases the fluidity thereby provides flexibility to the vesicles. The complete replacement of the phospholipid by the non-ionic surfactant also gives the bilayer vesicular nature and leads to introduction of another new class of vesiclular carrier called niosomes [56-59]. Van den Bergh introduced these surfactant based elastic vesicles and confirmed their effectiveness in the skin permeation enhancement. Cholesterol used in low concentrations as membrane stabilizing agent along with the surfactants caused rigidization of vesicles. Honeywell-Nguyen et al., investigated the niosomal delivery of D_2-agonist rotigotine and pergolide onto the skin [60. 61].

Admixture or full replacement of phospholipid in the liposomes resulted in the delivery of variety of novel vesicular carriers. In this series the usage of terpenes and ethanol with the phospholipid yields a novel carrier termed as invasomes [62. 63]. Terpenes are potential permeation enhancer, proved by the percutaneous permeation studies on the invasomes containing propranolol, lorazepam, clonazepam, haloperidol, tamoxofen etc. N.Dragicevic-Curic et al., used both ethanol and terpene with unsaturated phosphatidylcholine to prepare the invasomes containing temoporfin, a synthetic photosensitiser, named as invasomes. The *invitro* penetration studies showed a significantly higher permeation and skin deposition than the conventional liposomal formulation.

Apart from the vesicular carrier systems, other nanoparticulate carriers also made some advancement in transdermal delivery of drugs. Accordingly, Jongwon ship prepared minoxidil self assembled nanoparticles made up of poly (ε-caprolactone) –block- poly (ethyleneglycol) and evaluated the skin permeation using the skin samples of both hairless and hairy guinea pigs using franz diffusion cell [64]. The results revealed that the hairless skin have no *invitro* and *invivo* skin penetration, in contrast the hairy guinea pig which showed epidermal permeation. This indicated the shunt routes of hair follicles used as main route for delivery of the minoxidil block co-polymer nanoparticle. Instead of the polymers the substitution of solid lipids or liquid lipids leads to generation of another variety of nanoparticles called solid lipid nanoparticles. Lidocaine, halogenated glucocorticoids, titanium dioxide, 3,4,5- trimethoxy benzoylchtin for sunscreen activity, tretinoin, tropolide, imidazole antifungals, isotretinoin, ketoconazole, flurbiprofen, vitamin A were reported for dermal and transdermal delivery by nanostructured lipid carriers(NLC) and solid lipid nanoparticles(SLN) [65-70].

3. STRUCTURE OF ETHOSOMES (CONFIRMATION OF BILAYER CONFIGURATION)

E.Touitou et al., investigated the bilayer configuration of phospholipids and the effect of ethanol on bilayer configuration and permeability of the ethosomal phospholipid bilayer membrane [2]. ^{31}P-NMR studies were carried out on ethosomes containing 5% phospholipid, 20-50% of ethanol and water and liposomes containing same concentration of phospholipid without ethanol. The NMR spectrum of liposomes and ethosomes containing 30% ethanol and 50% ethanol were compared. The ethosomal system containing 30% ethanol showed the NMR peaks with a low field shoulder, which are typical for a phospholipid bilayer. The spectrum exhibited "solid state" line shape resembles like a large unsonicated liposomal dispersion. When the alcohol concentration was increased from 30-50 percentages, the chemical shift anisotropy was decreased from a higher field to lower field. Anisotropy is nothing but the shielding or deshielding effect on the proton due to the induced magnetic fields in other parts of the molecule which operate through space. Here the mobility of the polar head groups of phospholipids induced such a magnetic field and causes deshielding of the protons. Hence the shifting of signals from higher field to lower field has occurred. The chemical shift in ethosomal formulation is less than those obtained for liposomal formulation, showing that the mobility of the polar head groups in ethosomes is higher than in liposomes. The ethosomal formulation containing 50% ethanol showed a narrow and isotropic signal which indicates the absence of vesicular structure.

Further, the diffusion of ethosomal phospholipid bilayer membrane was investigated using Pr^{3+} as a shifting agent. If the ethosomal bilayer is impermeable to the cations when added externally after formation of vesicles will selectively shift the signals of phospholipids exposed to the external medium. If the vesicular membrane permits the cations, it will shift the signals until the inside and outside signals from phospholipids become merged. The results confirmed that in 20 to 30% ethanol concentration, the bilayers are closed in nature. But, in comparison to liposomes, ethosomes vesicles are characterized by a higher permeability for cations.

4. MECHANISM OF ENHANCED DRUG PERMEATION

The transdermal delivery of drug molecules from vesicular systems may possible by free drug penetration, permeation enhancement by vesicular components, intact vesicle penetration into and through the skin, vesicle adsorption and fusion with the stratum and pilosebaceous pathways.

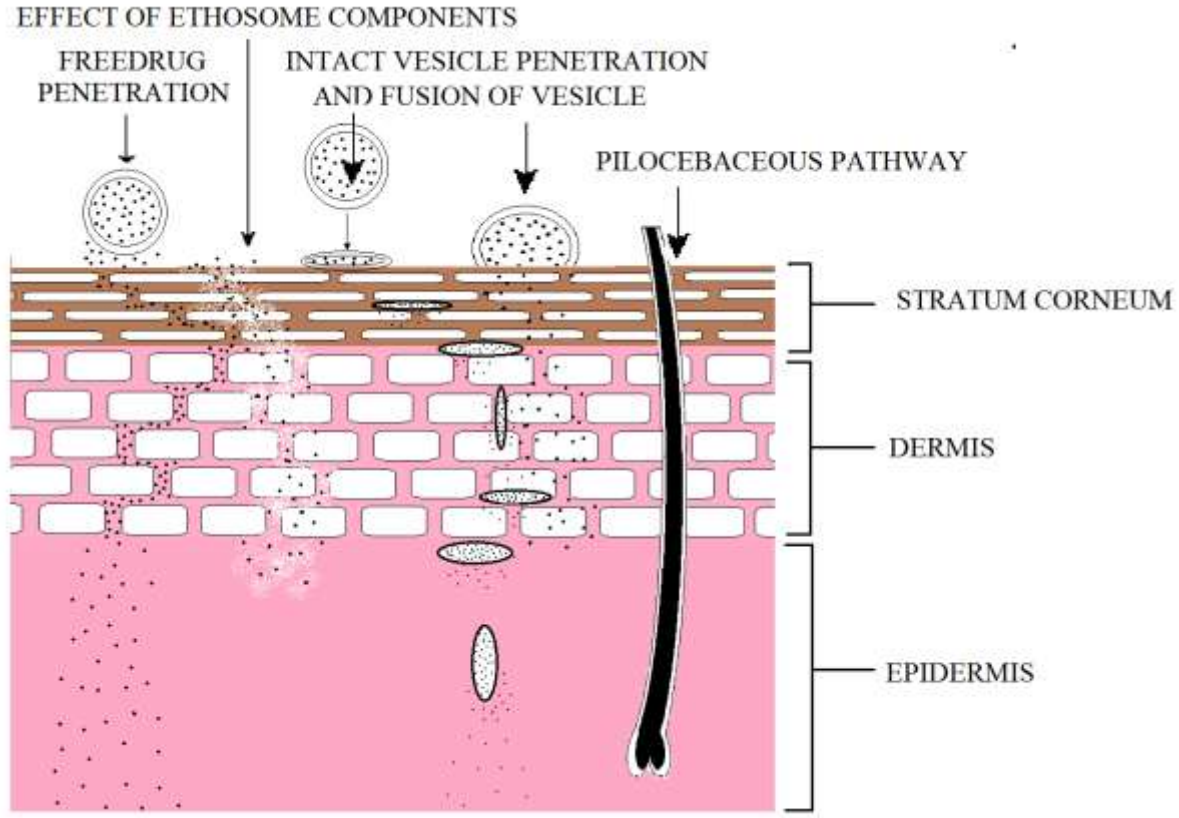

Fig. 2: An illustration explaining the various mechanisms exerted by the vesicular drug delivery systems including ethosomes (based on the diagram explained by El Maghraby) [71].

The evidence for permeation of vesicles through pilosebaceous pathways, penetration enhancement by ethosomal components, intact vesicle penetration into and through the skin and fusion of the vesicles with the skin were well proved by E.Touitou et al. One of the important component of the ethosomes, ethanol was a well known penetration enhancer in the drug delivery through the skin. Ethanol acts as a permeation enhancer in ethosomes by its fluidizing effect on the lipid bilayer membrane as well as on lipids of stratum corneum. In comparison to liposomes prepared without alcohol, the phosphatidyl choline in ethosomes is packed loosely. Thus ethosomal vesicles are more flexible when compare to the liposomes. The multi layers of stratum corneum are made up of compactly packed phospholipids which are the major permeation barrier. Ethanol disturbs the intact packing of the lipids and enhances its fluidity. The alteration of the construction of lipid bilayers is one of the supportive mechanisms in the enhanced drug delivery by ethosomes. The flexibility or fluidizing effect can be confirmed by calorimetric studies. The DSC studies were carried out by E.Touitou et al., for the ethosomal formulation containing 5% phospholipid and 30% ethanol and water and liposomes prepared without ethanol. DSC thermograms showed transition temperatures of -15.2 and 6.3 for ethosomes and liposomes respectively. The lower transition temperature(T_m) of ethosomes is due to fluidizing effect of ethanol which is absent in liposomes.

M.M.A.Elsayed et al., investigated the mechanism of enhanced skin delivery of ethosomes mainly depends on permeation enhancement effect of ethanol and flexibility of ethosomal vesicles [15]. The investigation is based on the comparison of *invitro* profiles of drug only inside the ethosomes, drug only outside the ethosomes and drug in both inside and outside the vesicle. Among them the ethosomal formulation containing the drug only inside the vesicle showed more permeation when compared the other two formulations. If the ethanol alone is an operating mechanism, the ethosomal formulation containing drug only outside the vesicle should also showed significantly enhanced penetration. The order of the amount of drug released from the formulation were drug only inside > drug in and out > drug only outside the vesicles. This result could suggest that the penetration enhancement effect of ethanol is not an operating mechanism. The flexibility of the vesicles plays a major role in the enhanced delivery of drug from ethosomes.

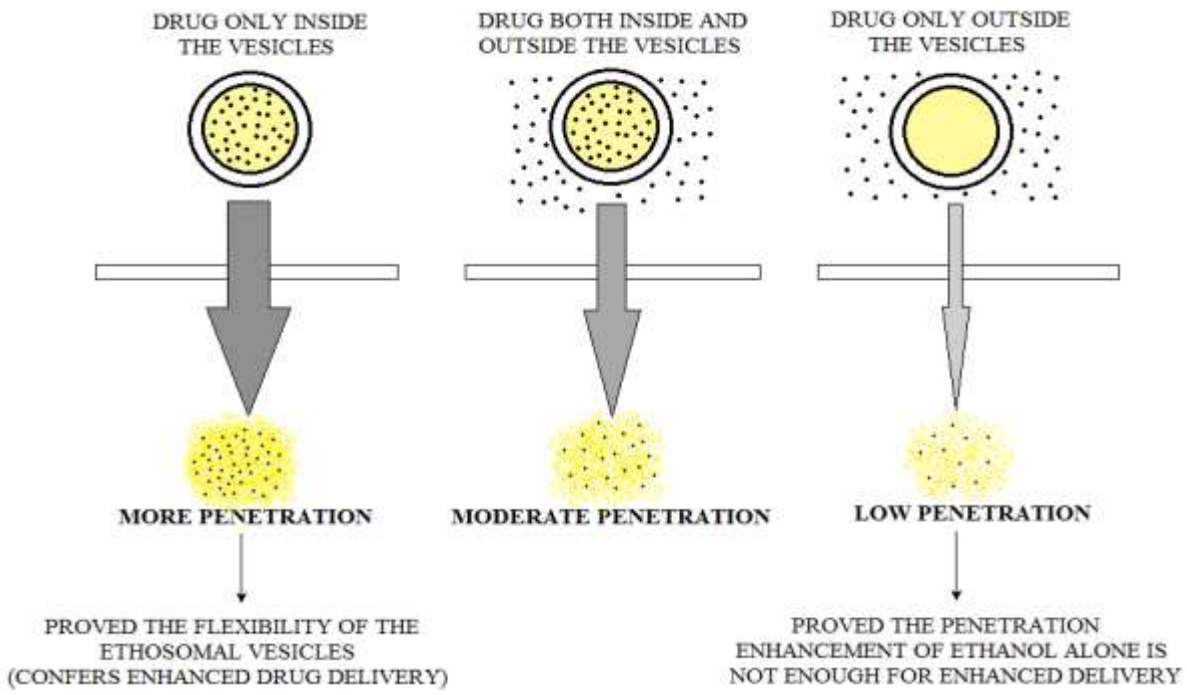

Fig. 3: A simple illustration that explaining the penetration enhancement of drug by the ethosomal vesicles

Vesicle adsorption and fusion with the layers of stratum corneum was proved by B.Godin and E.Touitou. Currently available antibiotics are not much more effective against intracellular infections due to their poor penetration and drug retention. They selected a polypeptide antibiotic bacitracin which acts against gram-positive bacteria. The liposomal and ethosomal formulation containing that drug bacitracin was prepared and the skin penetration behavior was studied using confocal lasser scanning microscope (CLSM). The active drug moiety from the liposomal formulation was not able to penetrate inside the cell. Liposomes undergo membranal adsorption with no drug penetration inside the cell. But from ethosomal formulation, in addition with intercalation of ethanol on stratum corneum lipids that causes increased membrane permeability, the fusion of flexible ethosomes with cell membrane and delivery of its components inside the cell was seen. Skin penetration profile obtained from CLSM studies showed the penetration of drug from the ethosomal vesicle structures peaked at ~40µm, while the depth of drug penetration was ~90µm, suggesting the fusion of ethosomes with stratum corneum lipid layers. The intracellular delivery of ethosomes was again confirmed by the same authors using fluorescent probe entrapped ethosomes on Swiss albino mice 3T3 fibroblasts.

5. APPLICATIONS OF ETHOSOMES

5.1 Applications of ethosomes as transdermal carrier

In the past ten years several studies were reported to prove the transdermal potential of ethosomes both by *invitro* and *invivo*. An exact comparison of drug permeation from two transdermal drug delivery systems containing ethosomes and plain drug was studied by E.Touitou [4]. Two transdermal patches containing testosterone, one purchased from market (Testoderm®) that contains plain drug and another prepared in laboratory that contains ethosomes named as testosome were compared for its transdermal delivery of drug both by *invitro* using franz diffusion cell, rabbit pinna skin as permeation barrier and *invivo* studies were carried out in male rabbits. The *invitro* skin permeation studies showed that the ethosome containing patches permeated 30 times greater than from testoderm® in 24 hours. Skin deposition was also favored by ethosomal patch, 7 times greater than testoderm®. Application of the ethosome containing patches *invivo* in rabbits for 5 days with a new patch applied every 24 hours showed 125% greater area under the curve than the commercially available testosterone patches. C_{max} was higher at any time with the ethosomal patch than the testoderm®. Another lipophilic drug minoxidil was formulated as ethosomes and *invitro* skin permeation studies were performed with the ethosomal suspension as such using side-by-side diffusion (Valia-

Chien) cells. The ethosomal formulation showed the cumulative amount of drug present in the receiver medium after *invitro* studies 10, 45 and 35 times more than the minoxidil solution in 2% phospholipid in ethanol, 30% hydroethanolic mixture and absolute alcohol respectively. Usually lipophilic drugs have more affinity with the skin than the hydrophilic one and ethanol act as a penetration enhancer for both of those categories. The combination of both lipophilic drug and ethanol should show enhanced permeation. As stated above even though testosterone and minoxidil are lipophilic drugs, the ethosomal formulation of these drugs showed enhanced skin permeation than its hydroethanolic solutions. Ethosomes containing trihexyphenidyl, a hydrophilic drug were also reported by the same author for its enhanced transdermal potential. Trihexyphenidyl is a cationic drug having pKa value of 8.7, ionisable in nature which causes poor skin permeation. Due to hydrophilic nature of the drug, it could not able to penetrate through the lipophilic stratum corneum, thereby transdermal delivery of this molecule is low. This drug was formulated as ethosomes and its transdermal ability was compared with liposomes, hydroethanolic solution and phosphate buffer using franz diffusion cell and male nude mouse skin as permeation barrier. In addition with the combined effect of ethanol as permeation enhancer and flexibility of ethosomal vesicles, encapsulation of drug in ethosomes renders more positive charge to its vesicle surface and favours higher *invitro* permeation. The flux of the drug from ethosomal vesicles was 87, 51 and 4.5 times greater than liposomes, hydroethanolic solution and phosphate buffer respectively. The skin deposition of drug was also significantly greater than the other formulations [3]. The results of these studies showed that the ethosomal formulations can significantly enhance the transdermal delivery of both hydrophilic and lipophilic drugs.

Transdermal enhancement of testosterone under nonpatch ethosomal application *invivo* was also reported by E.Touitou. An ethosomal formulation containing 1% testosterone, 50% ethanol, propylene glycol and phospholipon 90 was prepared and its *invivo* transdermal permeation behavior on Sprague-Dawley rats was compared with a currently used gel (AndroGel®) for testosterone replacement therapy. Blood samples were collected after single dermal application of both the formulations and drug concentration at each collection was determined using radio immuno assay. The results of blood samples showed significantly higher C_{max} and AUC for ethosomal formulation. Ethosomal formulation showed C_{max} more than 3 times and AUC nearly 2 times as that of the marketed gel. Further, *invitro* permeation studies through human skin were performed for theoretical estimation of testosterone concentration in blood following ethosomal application. The amount of testosterone permeated through the skin after 24hours of ethosomal application was more than 6 times than the marketed gel formulation. The theoretical estimation conclude $400cm^2$ and $40cm^2$ skin surface area is need to attain the required plasma concentration of the hormone for marketed gel and ethosomal formulation respectively. Ethosomal formulation requires 10 times lesser skin surface area than AndroGel. The higher pharmacokinetic parameters like C_{max} and AUC *invivo* and higher cumulative drug permeation *invitro* causes smaller application area and reduced dose by the application of ethosomal formulation and improves patient compliance [6].

The potentiality of ethosomes as transdermal drug delivery system *invivo* was successfully proved with its plama concentration time profile, skin deposition, organ distribution and *invivo* pharmacodynamic studies by M.Lodzki [5]. Cannabidiol, a new drug molecule useful in rheumatic diseases was selected for transdermal delivery via ethosomes, because of its low oral bioavailability (only 6% in humans), high first pass hepatic metabolism and incomplete gastrointestinal absorption due to its high lipophilicity and low aqueous solubility. Transdermal delivery of this drug candidate was also problematic one due to its lipophilicity which causes deposition of drug only at stratum corneum layer of the skin. This drug was formulated as ethosomes, incorporated in a carbomer gel base and deposition of the drug in abdominal skin, hip skin, and muscle beneath the application sites, pancreas and liver after 24 hours of occluded applications was determined. The plasma concentration profile for 72 hours and anti-inflammatory effect for 4 hours after application of the ethosomal gel was evaluated. Significant amount of the drug on skin was identified by HPLC and trace amount of drug in the muscles, liver and pancreas was identified by GC-MS after homogenization and extraction of the drug with organic solvents. The evaluation of plasma concentration profile states that the steady state levels were attained after 24 hours and maintained until the end of the experiment. The transdermal dose of the drug after 12 and 73 hours of application was calculated as 22.83 and 43.33% respectively. The pharmacodynamic character, anti-inflammatory effect was evaluated by carrageenan induced paw edema method. Carrageenan exhibits a time dependent increase in paw volume, which shows significant increase after 1 hour. Cannabidiol ethosomal formulation pre-treated mice showed significantly higher anti-inflammatory effect than the non-pretreated mice starting the drug application from 1 hour post carrageenan injection. The presence of drug in skin,

underlying muscle, liver, pancreas, construction of plasma concentration profile and *invivo* anti-inflammatory activity proved the ethosomes as a potential transdermal carrier [5].

A brief summary of other research works to prove transdermal potential of ethosomes

S.No	Reason for ethosomal design	Study design	Results
1.	Melatonin [16] (a neurohormone, mediate various cellular and physiological processes, having free radical scavenging activity) ➢ Very short plasma half life ➢ Variation in plasma half life ➢ Extensive first pass metabolism ➢ Poor skin permeation ➢ Long lag time on transdermal administration	➢ Ethosomes and liposomes were prepared by injection to ethanolic solution method and classic mechanical dispersion method respectively ➢ Fluorescent loaded ethosomes was also prepared to evaluate the depth of skin penetration by CLSM ➢ Skin permeation behaviour from ethosomes, liposomes, hydroethanolic solution of drug and plain drug solution were compared *invitro* using franz diffusion cells ➢ FT-IR studies of human cadaver skin after 6 hours treatment of ethosomes, liposomes and hydroethanolic solution ➢ Comparative skin irritation potential studies of ethosomes, hydroethanolic solution and plain drug solution	➢ Ethosomal formulation showed several times higher cumulative percentage of drug release than the other formulations ➢ Ethosomes showed 3 times and 6 times more flux than the hydroethanolic solution and conventional liposomes ➢ Ethosomes showed 2 and 3 times lesser lag time than the hydroehtanolic solution and liposomes ➢ FT-IR studies revealed high fluidization effect of ethosomes ➢ CLSM studies proved the higher depth of skin penetration ➢ No irritation (erythema) produced by ethosomes.
2.	Lamivudine [17] (a hydrophilic antiretroviral drug commonly used in AIDS and hepatitis) ➢ Short biological half life ➢ Frequent administration is required for a prolonged period of time	➢ *Invitro* skin permeation of ethosomes was compared with liposomal formulation, drug in 2% ethanolic phospholipid solution, 45% hydroethanolic solution of drug, ethanol solution of drug and phosphate buffer saline drug solution	➢ The flux of ethosomes was 5 fold higher than liposomes, 8 fold higher than drug in 2% ethanolic phospholipid solution,15 fold higher than 45% hydroethanolic solution of drug, 12 fold higher than ethanol solution of drug and 25 fold higher than phosphate buffer saline drug solution
3.	Solbutamol sulphate [18] (a water soluble drug used in the treatment of chronic bronchitis, bronchial asthma and emphysema) ➢ Readily absorbed from GI tract but undergoes extensive first-pass hepatic metabolism and gut wall metabolism ➢ Short plasma half life estimated 4 to 6 hours, require dose administration every 4 to 6 hours	➢ Liposomal formulation containing only cholesterol as membrane stabilizing agent was prepared thin film hydration method ➢ Liposomal formulation containing optimum concentration of cholesterol was selected and increasing concentration of dicetyl phosphate as liposomal membrane charge inducer ➢ Optimum concentrations of phosphatidyl choline,	➢ As increasing concentration of cholesterol caused decreasing drug release ➢ Dicetyl phosphate decreases the drug release, however an optimum concentration of dicetyl phosphate was used for further studies because of its higher skin deposition charactor ➢ Ethosomal formulation having 40%vol/vol alcohol concentration showed higher drug release was selected as

		cholesterol and dicetyl phosphate were used in the preparation of ethosomal formulation with increasing concentration of ethanol ➢ An optimized ethosomal formulation incorporated in pluronic F 127 gel and its permeation behaviour was studied	best formulation and incorporated in gel base. ➢ Gel formulation having 20% wt/wt pluronic F127 was finalized as best formulation because of its closeness to achieving required therapeutic concentration
4.	Matrine ethosome [25] (sophora flavescens alkaloid, applied for various inflammatory dermatoses, arthritis and tumors) ➢ Short plasma half life ➢ Low stability in formulations ➢ Faster degradation ➢ Pain and inflammation at the site of injection ➢ Oral formulations are susceptible to easy oxidation and discolouration during preparation and storage	➢ *Invitro* percutaneous permeation of drug from ethosomes was compared with hydroethanolic solution of drug and aqueous solution of drug ➢ Skin tolerability for empty ethosomes was evaluated by possible erythema induction ➢ *Invivo* anti-inflammatory activity of ethosomal formulation was evaluated by disappearance of erythema induced by methyl nicotinate and compared with hydoethanolic, water and saline solution of the drug.	➢ Drug permeation from ethosomes was nearly 3 times and more than 7 times than shown by hydroethanolic solution and aqueous solution respectively ➢ Absence of erythema for empty ethosomes favours good skin tolerability ➢ Erythema was disappeared more rapidly and disappeared completely with ethosomal application than the other formulations
5.	Buspirone [27] (5-HT_{1A} receptor agonist, used in the treatment of the major menopausal syndrome) ➢ Rapidly absorbed in GI tract, but undergoes extensive first-pass metabolism ➢ Very short elimination half life ➢ Oral dosage form requires frequent dose administration ➢ It is a hydrophilic cationic molecule, does not possess enough skin permeability	➢ Depth of skin penetration was measured by CLSM ➢ *Invitro* skin permeation of drug from ethosomes was compared with aqueous systems ➢ Pharmacokinetic parameters like C_{max}, T_{max}, AUC and flux were determined ➢ Effect of ethosomal formulation on elevated tail skin temperature in rat model was evaluated ➢ Anxiolytic effect of ethosomes was measured in elevated T-maze anxiety rat model ➢ Skin samples after pharmacokinetic evaluations were analysed for any histological changes	➢ Ethosomes showed both more depth and intense fluorescence in the skin than the aqueous system ➢ Flux of drug release from ethosomes was 3 times more and low lag time than the aqueous system ➢ Ethosomal transdermal delivery showed non-significant difference with the oral dose in case of C_{max}, however showed more than 4 times higher AUC which indicates amount of drug ➢ Phamacodynamic evaluations of ethosomes showed significantly higher anxiolytic and decreased tail skin temperature than their control group

5.2 Applications of ethosomes for topical delivery of drugs

Adsorption and fusion of vesicles with the layers stratum corneum is one of the important mechanism of drug delivery from ethosomes, proved by B.Godin and E.Touitou. Among the other postulated mechanisms, this mechanism should play an important role in the topical delivery of drugs. CLSM studies were used to visualize depth and pattern of drug release from ethosomes. CLSM analysis of two skin samples after application of liposomes and ethosomes containing a fluorescent probe were analysed by these scientists and showed a slight fluorescent intensity at the topper most layer of skin with

the liposomes and fairly evenly distributed fluorescent intensity throughout the skin with the ethosomes. CLSM data indicated that the depth of ethosomal vesicles into the skin was peaked at ~40µm and the drug probe was peaked at ~90µm. These results revealed that deeper skin delivery was obtained with the ethosomes than the liposomes and also proved the fusion of vesicles with skin layers. Ethosomes delivers the fluorescent probe greater and deeper than the hydroethanolic solution, confirmed by E.touitou et al [4]. Skin delivery of minoxidil via ethosomes, 2%phosphaolipid solution in ethanol, 30% ethanolic solution and absolute ethanolic solutions were performed by the same author and the amounts of drug in receiver and in the skin were quantified. Ethosomes showed the amount of drug 10, 45 and 35 times in the receiver medium and 2, 7 and 5 times in the skin relative to 2%phosphaolipid solution in ethanol, 30% ethanolic solution and absolute ethanolic solution respectively.

Effect of cholesterol concentration on skin permeation of minoxidil from liposomes and ethosomes was studied by J.M.Lopez-Pinto [11]. CLSM studies revealed ethosomes possess more skin penetration ability than the liposomes. The incorporation of cholesterol in liposomes usually increases the rigidity of vesicles and makes them accumulate more in stratum corneum rather than deeper penetration. But the higher ethanol concentration in the ethosomes overcome the effect of cholesterol, maintains its fluidity and flexibility. Confocal laser scanning micrographs showed higher fluorescence intensity with the ethosomal formulation than obtained with the liposomal formulations. The ethosomal formulation having higher cholesterol and ethanol concentration showed intense fluorescence and deeper skin delivery of fluorescent probe. The results of *invitro* skin permeation studies showed 9.8 folds of flux from ethosomes relative to the liposomal formulation. Effect of minoxidil ethosomes on hair cycle of mice was performed four weeks after synchronization of hair cycle by removing the skin by animal hair clippers. Hairs well grew out of the skin at the day of 18 in the mice groups treated with minoxidil ethosomal formulations and slight black colour with plain minoxidil treated group. Moreover there was no erythema or oedema observed with the minoxidil ethosomes treated group [12].

A clinical evaluation of ethosomes containing acyclovir useful in the topical treatment of recurrent herpes labialis was carried out by Horwitz [7]. Labial herpetic infection is caused by herpes simplex virus type 1 and produces local pain, discomfort, cosmetic disfigurement and associated with psychosocial effects even after 10 to 14 days of healing. Topical acyclovir treatment is more advantageous than oral therapy, because of its poor intestinal absorption. A 5% acyclovir ointment was investigated in several clinical studies and showed minor clinical efficacy, because of its inadequate skin penetration. A comparative clinical efficacy of 5% ethosomal acyclovir formulation and marketed cream having same concentration showed significantly shorter time duration (1.6days) to crust formation in the patients treated with ethosomal formulation than the marketed cream (4.3days) and significantly shorter time duration (4.2 days) to loss of crust to the patients received ethosomal formulation than the marketed formulation (5.9 days).

Staphylococcus aureus is one of the causative pathogen in superficial and deeper skin infections. Systemic therapy of this pathogenic topical infection with most of the antibiotics including macrolides, β-lactams and other categories has been ineffective. Topical delivery of antibiotics is a best alternative to prevent severe allergic reactions and side effects of antibiotics used as oral dosage formulations. Erythromycin is a macrolide antibiotic that can be useful in many of dermal infections. But currently available topical antibiotic preparations are not effective to deliver the drugs into the deeper layers of skin. E.Touitou et al formulated erythromycin ethosomes, evaluated for its safety, bacterial inhibition ability *invitro* and *invivo* antibacterial activity against staphylococcus aureus (Biana Godin et al., 2005). Cultured dermal fibroblasts were used to determine the safety and showed no toxicity with the ethosomes and also proved there was no irritation and erythema. Agar well diffusion assay of erythromycin ethosomes showed higher inhibitory effect. It was also noted that ethosomal system without drug caused no inhibitory effect on bacterial growth, proved the antibacterial activity of erythromycin ethosomes was not caused by its ethanolic content. *Invivo* antibacterial activity supported that the ethosomal antibiotic formulations can be applied as an efficient carrier to treat the deep skin infections. 5-aminolevulinic acid is a drug molecule used in topical photo dynamic therapy, one of the treatment methods for non-melanoma skin cancer. Photo dynamic therapy requires 3 elements, a photo sensitizer, light irradiation and singlet oxygen. The photosensitizer is activated by light rays with suitable wavelength, destroying the tumour cells by necrosis or apoptosis and decreases the oxygen carrying capacity by preventing vascularisation. 5-aminolevulinic is photosensitizer precursor, having poor skin permeation due to its hydrophilic characteristic and charge. This drug was formulated as ethosomes by Y.P.Fang et al and its skin penetration property was determined by CLSM [23]. Ethosomal formulation showed intense and deep

penetration when compared to aqueous and hydroethanolic solution of drug. The skin irritation studies were also evaluated by colorimetry, showed no irritation. Anti-psoriatic effect of 5-aminolevulenic acid ethosomes was also reported by the same author, showed 5 and 26 fold in normal and hyperproliferative murine skin samples. *Invivo* CLSM studies proved higher skin penetration ability nearly 4 folds relative to its aqueous control. Temoporfin is also a photo sensitizer used in the treatment of skin carcinoma and psoriasis. Temoporfin ethanolic ethosomes having the maximum of 20% w/v of ethanol were prepared and showed increased skin deposition and highest skin penetration than the liposomal formulation [21]. Another anti-psoriatic agent methotrexate is reported by Vaibhav Dubey et al. Even though the drug is more hydrosoluble in nature, ethosomal system enhanced its transdermal flux nearly 4, 3 and 26 folds relative to liposomal formulation, hydroethanolic solution and plain drug solution respectively. Skin deposition of the ethosomes was also 3 times higher than the liposomal formulation which favors better psoriatic control.

Antifungal efficacy of fluconazole encapsulated ethosomes was evaluated both clinically and non-clinically by A.N.Mishra et al. Fluconazole is a broad spectrum antifungal antibiotic used in the treatment of candidacies and some other fungal skin infections. Clinical evaluation compared the antifungal efficacy that is reduction in dimension of skin lesions after application of fluconazole ethosomal gel, liposomal gel and marketed fluconazole cream and hydroethanolic solution. Among those four formulations ethosomal gel showed comparatively better reduction in dimension of lesions than the other 3 formulations. The antifungal efficacy was in the order of ethosomal gel formulation>liposomal gel formulation>marketed cream>hydroethanolic solution of the drug. *Invitro* skin permeation showed nearly double fold penetration from ethosomes than the liposomes [24]. Another antifungal drug miconazole was formulated as ethosomal ointment and simple *invitro* study was conducted to compare the release behavior of ethosomal, liposomal and plain ointment. In this study, ethosomal formulation showed 3.6 times and 1.2 times higher cumulative drug release than liposomal and plain ointment respectively [26].

5.3 Applications of ethosomes in intracellular delivery of drugs

The intracellular delivery of three fluorescent probes with various physiochemical properties was investigated on Swiss albino mice 3T3 fibroblasts by E.Touitou et al [8]. Intracellular delivery of fluorescent probe was evaluated by confocal lasser scanning microscopy and fluorescent-activated cell sorting and showed higher fluorescence intensity with ethosomal system than liposomes and hydroethanolic solution of fluorescent probes. An amphipathic fluorescent probe 4-(4-diethylamino) styryl-N-methylpyridinium iodide (D-289) and Rhodamine red-X were found throughout the cell including nucleus. Furthermore, viability test of the fibroblast cells showed that the ethosomal carrier was non-toxic to the cultured cells. Fluorescently labelled bacitracin and erythromycin were also formulated as ethosomes by the same author and the CLSM and FACS studies confirmed the intracellular delivery of both the antibiotics [13]. Intracellular and deeper skin penetration of α-tocopherol ethosomes was reported by E.Touitou [20] α-tocopherol is an antioxidant used in the prevention of skin malignancy, which requires intracellular delivery for optimal photoprotection [72]

6. CONCLUSION

Cure or suppression of the diseases like eczema, psoriasis, icthyosis and the skin cancers are noble aims of dermal and transdermal delivery of drugs. But the main reason for many problems associated with transdermal drug delivery is the barrier nature of human skin and limits the delivery of daily drug requirement. For example, from the accepted size of the patch approximately 10mg of drug only permeate into the skin. But the application of the ethosomal system in transdermal delivery will surely enhance the amount of drug permeated through the skin. Ethosomes can also permeate deeply and locally into the skin. Therefore, ethosomal formulations containing anticancer drugs can be formulated to treat deep seated tumors. Ethosomal transdermal delivery will definitely open a new window in the treatment of challenging skin diseases.

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