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ETHOSOMES: ANOVEL APPROACH TO DRUG

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INTRODUCTION

The skin is the largest and most easily accessible organ of the body; it serves as a potential route of drug administration for systemic effects. However, the outer layer of the skin, the stratum corneum, represents the most resistible barrier to drug penetration across the skin, which limits the trans-dermal bioavailability of drugs. Therefore, special carriers are required to combat the natural skin barrier to deliver drug molecules with different physicochemical properties to the systemic circulation.[1]

Trans-dermal drug-delivery systems offer many advantages, such as avoidance of first- pass metabolism by the liver, controlled delivery of drugs, reduced dosing frequency, and improved patient compliance, as they are noninvasive and can be self administered. The first Trans- dermal patch containing scopolamine for the treatment of motion sickness was approved in the US in 1979.[1,2,3]

A new era of research in this field was opened with the use of liposomes for the topicaldelivery of triamcinolone, and since then a wide range of novel lipid-based vesicular systems have been developed. Deformable or elastic liposomes, which are currently known as Transfersomes, were introduced by Cevc and Blume in 1924 and followed by the innovative work of Touitou et al, which led to the discovery of a novel lipid vesicular system called Ethosomes.[2]

Ethosomal systems differ from liposomes because they contain relatively high concentrations of ethanol, in addition to phospholipids and water. New generations of ethosomal systems have been introduced since then by adding other compounds to the basic Ethosomal formula in an attempt to enhance vesicular characteristics and skin permeation. However, to date, there has

been no clear distinction among the classical Ethosomes and their newer generations.[1]

This article provides a detailed review of Ethosomal systems and identifies the different types ofthese vesicles based on the compounds used in their production and the impact of these compounds on Ethosomal properties. Additionally, this article also highlights the Ethosome preparation methods and pharmaceutical dosage forms, as well as the in vivo studies and clinicaltrials conducted on these promising nanocarr iers for dermal/transderm.

ROUTES OF PENETRATION

At the skin, molecules contact cellular debris, microorganisms, sebum and other materials, which negligibly affect permeation. The penetrant has three potential pathways to the viable tissue - through hair follicles with associated sebaceous glands, via sweat ducts, or across continuous stratum corneum between these appendages.[2,3]

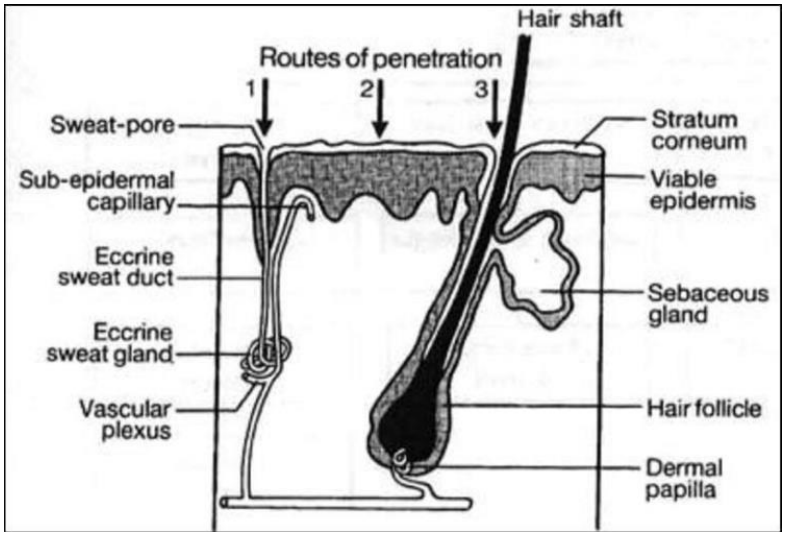

Figure 1: Simplified diagram of skin structure and macroroutes of drug penetration (1) via the sweat ducts; (2) across the continuous stratum corneum or (3) through the hair follicles with theirassociated sebaceous glands.

Rational appendageal area available for transport is only about 0.1%; this route usually contributes negligibly to steady state drug flux. The pathway can be important for ions and largepolar molecules that struggle to cross intact stratum corneum. Appendages may be providing shunts, important at short times prior to steady state diffusion. Additionally, polymers and colloidal particles can target the follicle.[2,3]

The intact stratum corneum thus provides the main barrier; its 'brick and mortar' structure is

analogous to a wall. The corneocytes of hydrated keratin comprise of 'bricks', embedded in 'mortar', composed of multiple lipid bilayers of ceramides, fatty acids, cholesterol and cholesterol esters. These bilayers form regions of semi-crystalline, gel and liquid crystals domains. Most molecule penetrate through skin via this intercellular microroute and therefore many enhancing techniques aim to disrupt or bypass elegant molecular architecture. Viable layers may metabolize a drug, or activate a prodrug. The dermal papillary layer is so rich in capillaries that most penetrants clear within minutes. Usually, deeper dermal regions do not significantly influence absorption, although they may bind e.g.

Testosterone, inhitibiting its systemic removal.[1,2,3,4]

OPTIMISING TRANSDERMAL DRUG DELIVERY

Transdermal route offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing under able side effects, utility of short half- life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter and intra patient valuations, and most importantly, it provides patient convince. But one of the major problems in transdermal drug delivery is the low penetration rate through the outer most layer of skin.[2,4]

The non-invasive approaches for providing transdermal drug delivery of various therapeutic substances are:[1,2]

1) Stratum corneum modification

a) Hydration

b) Chemical penetration enhancers

2) Stratum corneum bypassed or removed

a) Microneedle array

b) Stratum corneum ablated

c) Follicular delivery

3) Electrically assisted methods

a) Ultrasound (Phonophoresis, Sonophoresis)

b) Iontophoresis

c) Electroporation

d) Magnetophoresis

4) Vesicles and particles

a) Liposomes and other vesicles

b) Niosomes

c) Ethosomes.[1,2,3,4]

1. Ultrasound (Phonophoresis, Sonophoresis)

Phonophoresis, also known as sonophoresis or ultrasonophoresis, is when an ultrasound is used to maximize the effects of a topical drug. The ultrasound waves push particles of a pain-relieving or anti-inflammatory drug deeper into your skin tissues. When these particles reach the subcutical layer, the deepest layer of skin, the cells absorb the pain reliever.[2]

2. Iontophoresis

Iontophoresis is a process of transdermal drug delivery by use of a voltage gradient on the skin. Molecules are transported across the stratum corneum by electrophoresis and electro-osmosis and the electric field can also increase the permeability of the skin.

Mainly used to deliver Pain killers or Anti-Diabetic drugs.[2,3]

3. Electroporation

Electroporation, or electropermeabilization, is a microbiology technique in which an electrical field is applied to cells in order to increase the permeability of the cell membrane, allowing chemicals, drugs, electrode arrays or DNA to be introduced into the cell (also called electrotransfer).[3]

4. Magnetophoresis

Magnetophoresis is the term used to describe the enhancement of drug permeation across a biological barrier by the application of a magnetic field.[3,4]

5. Liposomes

A minute spherical sac of phospholipid molecules enclosing a water droplet, especially as formed artificially to carry drugs or other substances into the tissues.[2,3,4,5]

6. Niosomes

A niosome is a non-ionic surfactant-based vesicle**.** Niosomes are formed mostly by non- ionic surfactant and cholesterol incorporation as an excipient. Other excipients can also be used.[4]

7. Ethosomes

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.[4,5]

ETHOSOMES

Ethosomes are Phospholipid, nanovesicles used for dermal and transdermal delivery of molecules. Ethosomes were developed by Touitou et al., 1997, as additional novel lipid carriers composed of ethanol, phospholipids, and water. They are reported to improve the skin delivery of various drugs. Ethanol is an efficient permeation enhancer that is believed to act by affecting the intercellular region of the stratum corneum. Ethosomes are soft malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration), and water. These soft vesicles represent novel vesicles carriers for enhanced delivery through the skin. The size of the ethosomes vesicles can be modulated from tens of nanometers to microns.[4,5]

The vesicles have been well known for their important in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicle structures for use in better drug delivery within their cavities, that would allow to tag the vesicle for cell specificity. Vesicles would also allow to control the release rate of drug over an extendedtime, keeping the drug shielded from immune response or other removal systems and would be able to release just the right amount of drug and keep that concentration constant for longer periods of time. One of the major advances in vesicle research was the finding a vesicle derivative, known as an ethosomes. Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipid (Phosphotidyl choline;, ethanol at relatively high concentration and water. It was found that ethosomes penetrate the skin and allow enhanced delivery of various compounds to the deep strata of the skin or to thesystemiccirculation.[4,5]

Structure and Composistion of Ethosomes

Ethosomes are soft malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration), and water. These soft vesicles represent novel vesicles carriers for enhanced delivery through the skin.[5]

Ethosomes are mainly composed of multiple, concentric layers of flexible phospholipid bilayers, with a relative high concentration of ethanol (20-45%), glycols and water. Their

overall structure has been confirmed by 31P-NMR, EM and DSC. They have high penetration of the thorny layer of the skin, which enhances the permeation of encapsulated drugs. The mechanism of permeation enhancement is attributed to the overall properties of the system.[4,5,6,7]

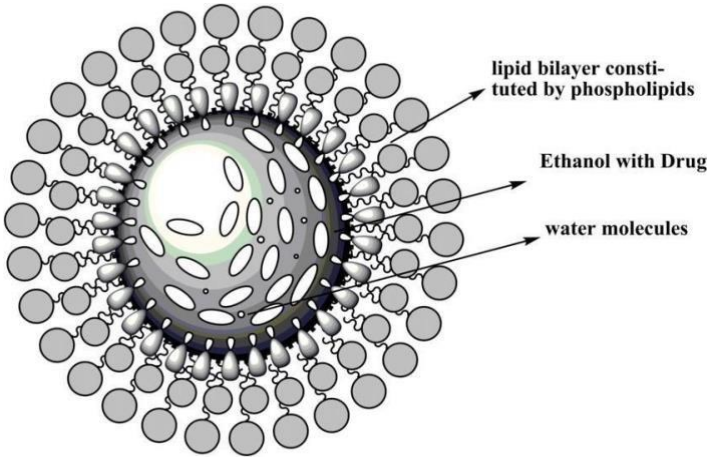

Fig. No. 02: Structure of Ethosomes.

The Ethosomes are vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. Typically, ethosomes may contain phospholipids with various chemical structures like phosphatidylcholine (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PPG), phosphatidylinositol (PI), water and propylene glycol (or other glycols). Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: waterratio. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5- 10% w/w. Cholesterol at concentrations ranging between 0.1-1% can also be added to the preparation. Examples of alcohols, which can be used, include ethanol and isopropyl alcohol. Among glycols, propylene glycol and Transcutol are generally used. In addition, non-ionic surfactants (PEG- alkyl ethers) can be combined with the phospholipids in these preparations. Cationic lipids like cocoamide, POE alkyl amines, dodecylamine, cetrimide etc. can be added too. The concentration of alcohol in the final product may range from 20% to 50%. The concentration of the non- aqueous phase (alcohol and glycol combination) may range between 22% to 70%.[4,5]

Table 1: Different Additives Employed In Formulation of Ethosomes.

Class	Example	Uses
Phospholipid	Soya Phophotidyl choline, Egg Phosphotidyl Choline, Dipalmityl Phosphotidyl Choline, Distearyl Phosphotidyl Choline.	Vescicle forming component.
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer.
Alcohol	Ethanol Isopropyl glycol	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

ETHOSOMAL SYSTEM TYPES

1. Classical Ethosomes

Classical Ethosomes are a modification of classical liposomes and are composed of phospholipids, a high concentration of ethanol up to 45% w/w, and water. Classical Ethosomes were reported to be superior over classical liposomes for transdermal drug delivery because 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.6–8. The molecular weights of drugs entrapped in classical Ethosomes have ranged from 130.077 Da to 24 kDa.[5,6]

1. Binary Ethosomes

Binary Ethosomes were introduced by Zhou et al.11 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).

2. Trans-Ethosomes

Transethosomes are the new generation of ethosomal systems and were first reported by Song et al in 2012. This ethosomal system contains the basic components of classical ethosomes and an additional compound, such as a penetration enhancer or an edge activator (surfactant) in their formula. These novel vesicles were developed in an attempt to combine the advantages of classical ethosomes and deformable liposomes (transfersomes) in one formula to produce Transethosomes. Many researchers have reported superior properties of transethosomes over

classical ethosomes.17–30 Different types of edge activators and penetration enhancers have been investigated to produce ethosomal systems with better characteristics. Transethosomes were reported to entrap drugs with molecular weights ranging from 130.077 Da to 200–325 kDa.[4,5,6,7]

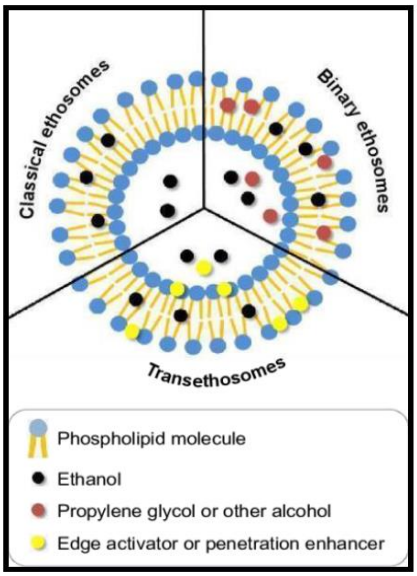

Fig. 3: Schematic representation of the different types of Ethosomal systems.

MECHANISM OF PENETRATION OF ETHOSOMES

Although the exact process of drug delivery by Ethosomes remains a matter of speculation, most likely, a combination of processes contributes to the enhancing effect. The stratum corneum lipid multilayer at physiological temperature are densely packed and highly conformationally ordered. The high concentration of ethanol makes the Ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayers organization; therefore, when integrated into a vesicle membrane, it gives that vesicles have the ability to penetrate the stratumcorneum. Also because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles but has equivalent stability, allowing a more malleable structure, giving it more freedom and ability to squeeze through small places such as the openings created in disturbing the stratum corneum lipid.[5,6,7]

Ethanol interacts with lipid molecules in the polar hard group region, resulting in a reducting the rigidity of the stratum corneum lipids, increasing their fluidity. The intercalation ofethanol into the polar head group environment can result in an increase in the membrane permeability. In addition to the effect of ethanol on stratum corneum structure, the Ethosome itself may interact with the stratum corneum barrier.[6]

The interdigitated, malleable Ethosome vesicle can forge paths in the disordered stratum corneum. In the case of Ethosomes encapsulating drugs, the higher positive zeta potential imparted by the drug can improve skin attachment of the vesicles. While encapsulated drug in classic liposomes remained primarily at the surface of the skin the ethosomal system was Showed to be highly efficient carrier for enhanced drug delivery through the skin. The efficient drug delivery shown together with the long-term stability of Ethosomes make this system a promisingcandidate for transdermal delivery of drug.[6,7]

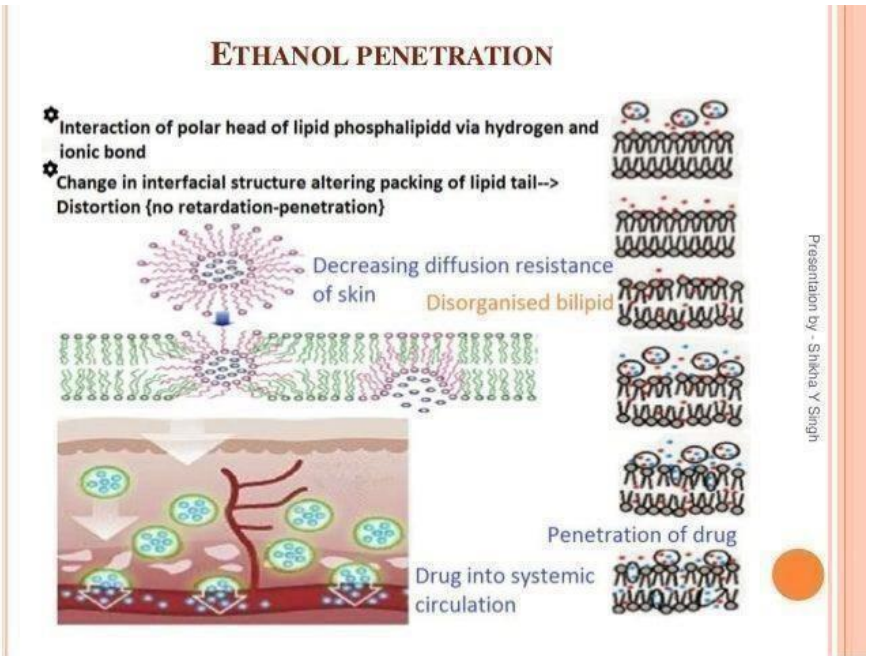

Fig. No. 03: Mechanism of Penetration of Ethosomal System.

METHOD FOR PREPARING ETHOSOMES

1. *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 300C in a water bath. The water heatedto 300C 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 or extrusion method. Finally, the formulation is stored under refrigeration.[7,8]

2. HOT METHOD

In this method phospholipid is dispersed in water by heating in a water bath at 400C until a

colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400C. Once both mixtures reach 400C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties .The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.[8]

3. *THE THIN-FILM HYDRATION METHOD*

This represents the extension of the conventional liposome preparation method, but in this method the lipid film is hydrated by a hydro-ethanolic solution. The Phospholipid is first dissolved in chloroform only85 or a chloroform–methanol mixture at ratios of 3:110 or 2:186 in a clean, dry, round-bottom flask. Organic solvents are removed by a rotary vacuum evaporator ata temperature above the lipid-phase transition temperature. Then, the traces of the solvents are removed from the deposited lipid film under vacuum overnight. The lipid film is then hydrated with a water–ethanol solution or phosphate buffered saline–ethanol solution.87 During the hydration process, the lipid film is rotated and heated at the required temperature, which dependson the phospholipid property, for 30 minutes, 1 hour or 6 hours.

4. *THE REVERSE-PHASE EVAPORATION METHOD*

This is the least used method and specially designed to produce large unilamellar vesicles. The organic phase is prepared by dissolving the phospholipid in diethyl ether and then mixing itwith the aqueous phase at a ratio of 3:1 v/v in an ultrasonic bath at 0°C for 5 minutes to form a water-in oil emulsion. The organic solvent is removed under reduced pressure to produce a gel, which turns into a colloidal dispersion upon vigorous mechanical agitation.[8,9]

In Vivo Studies

In vivo studies highly diverse in vivo studies and models have been carried out by many researchersusing humans, rabbits, rats, mice, and guinea pigs for the evaluation of ethosomal systems involving skin permeation, pharmacokinetics, pharmacodynamics, safety, and skin irritation studies. The ability of ethosomal systems to penetrate intact skin, delivering molecules with diverse physicochemical properties in therapeutic amounts to the blood circulation, has been reported by several in vivo studies.[8,9,10]

For instance, Ahad et al studied the pharmacokinetic and pharmaco-dynamic effects of a Valsartan- loaded Ethosomal system in Albino Wistar rats. The bioavailability of transdermal ethosomal Valsartan was significantly higher (3.03 times) than the oral Valsartan suspension;

the area under the concentration–time profile curve from time 0 to ∞ for the transdermal ethosomal formulation was 177,298.82±665.01 ng/mL/h, while for the oral suspension it was 55,554.54±774.01 ng/mL/h. The dose of Valsartan used in both routes was 3.6 mg/kg. The reported maximum drug concentration (Cmax) and the time taken to reach maximum concentration (Tmax) values for the oral administration were 13,100±101.12 ng/mL and 1±0.01 hours, respectively. The Cmax and Tmax values for the transdermal route were 7,944±134.32 ng/mL and 5.0±0.83 hours, respectively. Ethosomal valsartan was shown to effectively reduce blood pressure by 34.11% for 48 hours in hypertensive rats.[7,8]

In vivo safety studies on humans and animals have shown that classical ethosomes are highly safe with excellent skin tolerability. Further in vivo safety studies are much required to evaluate the short- and long-term effects of repeated application of these nanocarriers on the skin, particularly for the other two classes of ethosomal systems (binary ethosomes and transethosomes), which contain penetration enhancers and/or edge activators in their formulation and thus may increase the possibility of developing adverse skin reactions, such as irritations or erythema.[7]

Clinical trials

Based on a literature searches, only three clinical trials have been conducted on ethosomal systems in human volunteers (Table 6). 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.135 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. Ethosomal preparation of prostaglandin E1 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 wa.

ETHOSOMAL DOSAGE FORMS

The majority of the published articles have studied ethosomal systems in their initial

suspension form. Ethosomal suspension contains a high concentration of alcohol, and thus further incorporation of the system in a suitable vehicle for dermal/transdermal delivery has some advantages, ie, preventing ethanol evaporation, prolonging contact time with the skin, enhancingthe therapeutic efficacy of the entrapped drug, improving stability and shelf life of the final dosage form, and patient compliance. Ethosomal systems have been incorporated in different vehicles to produce novel pharmaceutical formulations, such as Ethosomal Gels, Transdermal Patches, and Creams.[9]

1) Ethosomal Gels

Ethosomal gels are characterized for their pH, viscosity, spreadability, and extrudability. The most commonly used gel-forming agents for incorporating ethosomal systems are Carbopoland hydroxypropyl methylcellulose with all their related grades. These polymers have been shown to be compatible with ethosomal systems, providing the required viscosity and bioadhesive properties.[7,8,9]

Several researchers have studied the skin-permeation and disposition properties of drugs from Ethosomal gels in comparison to the traditional or marketed gels or creams. Puri and Jain compared the in vitro skin-permeation properties of 5-fluorouracil from Ethosomal gel and marketed cream using Franz diffusion cell and albino rat skin and found that the transdermal fluxof 5-fluorouracil from Ethosomal gels was 4.9-fold higher than the marketed cream. Moreover, the skin disposition of the drug from the Ethosomal gel was 9.4-fold higher than the marketed cream. Other researchers found that in vitro transdermal flux of Aceclofenac from Ethosomal gelwas higher (226.1 µg/cm2 /h) than Zynac gel (131.1 µg/cm2 /h). Some authors have reported the same superior properties of Ethosomal gels for different drugs/agents over traditional gels.

Interestingly, it was found that the drug-release rate from the ethosomal suspension was faster than from Ethosomal gel, due to the high viscosity of the gel.[8,9]

2) Ethosomal patches

The preparation and evaluation of ethosomal patches are more complicated than for ethosomal gels, as molds are required for their preparation. Based on a literature search, only seven research articles had reported ethosomal patch formulations for several drugs, i.e, testosterone, Artesunate and Febrifugine, Ligustrazine, Valsartan, Tizanidine hydrochloride, andinsulin. 120 Different polymers were used to prepare the Ethosomal patches, including

Polyvinylpyrrolidone/Vinyl acetate, acrylic resin, and hydroxypropyl methylcellulose.

Triethyl citrate was added to the formulation as a plasticizer.[9]

Touitou et al compared the in vitro and in vivo transdermal delivery properties from Ethosomal patches and the non-ethosomal patches of testosterone marketed as Testoderm; both patches had the same dimensions and drug contents. In vitro studies were done using Franz diffusion cells and rabbit pinna skin taken from the ear. The results showed that 24 hours after application, the amount of testosterone permeated from the ethosomal patches was 30 times higher (848.16±158.38 µg) than the Testoderm patches (27.79±16.23 µg), while drug depositionin the skin was seven times higher from the ethosomal patch than the Testoderm patch.[8,9]

3) Ethosomal creams

There have only been two studies reporting the formulation of Ethosomal creams. Both of these involved the incorporation of Curcuma Longa extract-loaded Ethosomal systems in a cream base as a photo- protective and anti-wrinkle agent. In both studies, C. Longa extract-loadedEthosomal creams were applied to human volunteers and showed promising results as either a photoprotective or an anti- wrinkle agent. Based on all the aforementioned studies, the incorporation of Ethosomal systems in suitable vehicles such as gels, patches, and creams improves skin permeation of the drug/ agent from the Ethosomal systems. Among the vehicles discussed, gels are the most suitable vehicle for the incorporation of Ethosomal systems, while Ethosomal creams may be preferred for cosmetic preparations.[8]

PILOSEBACEOUS TARGETING

Hair follicles and sebaceous glands are increasingly being recognized as potentially significant elements in the percutaneous drug delivery. Interest in Pilosebaceous units has been directed towards their use as depots for localized therapy, particularly for the treatment of follicle- related disorders such as acne or alopecia.[8]

Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery. With the purpose of Pilosebaceous targeting, Maiden et al. prepared and evaluated Minoxidil ethosomal formulation. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness. Conventional topical formulation has very poor skin permeation and retention properties. It was found that the

quantity of Minoxidil accumulated into nude mice skin after application of its Ethosomal formulation was 2.0, 7.0 and 5.0 fold higher as compared to ethanolic phospholipids dispersion, Hydro-ethanolic solution and Ethanolic solution of drug each containing 0.5% of the drug.[9]

APPLICATIONS

Table No. 02: Results of Drugs used as an Ethosomal system of carrier.

SR.NO.	DRUG	RESULTS
1	NSAIDS (Diclofenac)	1. Selective delivery of drug to desired side for prolong period of time.
2	Acyclovir	1. Increase skin permeation. 2. Improved in biological activity 2-3 times. 3. Improved in Pharmaco-dynamic profile.
3	Insulin	1. Significant decrease in blood glucose level. 2. Provide control release.
4	Trihexyphenidylhydrochloride	1. Improved transdermal flux. 2. Provide controlled release. 3. Improved patient compliance. 4. Biologically active at dose several times lower than the currently used formulation.
5	Antibiotic Cannabinol Erythromycin	1. Improved skin deposition. 2. Improved biological activity. 3. Prolonging drug action.
6	Anti-HIV agents Zidovudine Lamivudine	1. Improved transdermal flux 2. Improved inbiological activity 2-3times. 3. Prolonging drug action. 4. Reduced drug toxicity. 5. Affected the normal histology of skin.
7	Azelaic acid.	1. Prolong drug release.
8	Ammonium glycyrrhizinate	1. Improved dermal deposition exhibiting sustained release. 2. Improved biological anti- inflammatory activity.

TRANSDERMAL DELIVERY OF HORMONES

Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several dose dependent side effects. In addition, along with these side effects oral hormonal preparations relying highly on patient compliance. The riskof failure of treatment is known to increase with each pill missed.[8,9,10]

Touitou et al. compared the skin permeation potential of testosterone ethosomes (Testosome) across rabbit pinna skin with marketed transdermal patch of testosterone (Testoderm¨ patch, Alza). They observed nearly 30-times higher skin permeation of testosterone from ethosomal formulation as compared to that marketed formulation. The amount of drug deposited was

significantly ($p<0.05$) higher in case of ethosomal formulation (130.76 -18.14 and 18.32 - 4.05 g at the end of 7 hr for Testosome and Testoderm¨, respectively).

The AUC and Cmax of testosterone significantly improved after application of Testosome as compared to Testoderm. Hence, both in vitro and in vivo studies demonstrated improved skin permeation and bioavailability of testosterone from ethosomal formulation. Thisgroup in their further study designs the testosterone nonpatch formulation to reduce the area of application.[101] They have found that with ethosomal testosterone formulation area of application required to produce the effective plasma concentration was 10 times less than required by commercially gel (AndroGel¨) formulation.[9]

DELIVERY OF ANTI-PARKINSONISM AGENT

Dayan and Touitou prepared ethosomal formulation of psychoactive drug trihexyphenidyl hydrochloride (THP) and compared its delivery with that from classical liposomal formulation. THP is a M1 muscarinic receptors antagonist and used in the treatment of Parkinson disease. THP has a short biological half-life (3hr) 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 microscope found to consist of small, phospholipid vesicles. The value of transdermal flux of THP through nude mouse skin from ethosomes was 87, 51 and 4.5-times higher than that from liposome, phosphate buffer and hydroethanolic solution, respectively. The quantity of THP remaining in skin at the end of 18 hr was significantly higher after application of ethosomes than after application of liposome or hydroethanolic solution (control). These results indicated better skin permeation potential of ethosomal-THP formulation and its use for better management of Parkinson disease.[9]

TOPICAL DELIVERY OF DNA

Many environmental pathogens attempt to enter the body through the skin. Skin therefore, has evolved into an excellent protective barrier, which is also immunologically active and able to express the gene. On the basis of above facts another important application of ethosomes is to use them for topical delivery of DNA molecules to express genes in skin cells. Touitou et al. in their study encapsulated the GFP-CMV-driven transfecting construct into ethosomal formulation. They applied this formulation to the dorsal skin of 5-week male

CD-1 nude mice for 48 hr. After 48 hr, treated skin was removed and penetration of green fluorescent protein (GFP) formulation was observed by CLSM. It was observed that topically applied ethosomes- GFP-CMV-driven transfecting construct enabled efficient delivery and expression of genes in skin cells. It was suggested that ethosomes could be used as carriers for gene therapy applications that require transient expression of genes. These results also showed the possibility of using ethosomes for effective transdermal immunization. Gupta et al. recently reported immunization potential using transfersomal formulation. Hence, better skin permeation ability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents

DELIVERY OFANTI-ARTHRITIS DRUG

Topical delivery of anti-arthritis drug is a better option for its site-specific delivery and overcomes the problem associated with conventional oral therapy. Cannabidol (CBD) is a recently developed drug candidate for treating rheumatoid arthritis. Its oral administration is associated with a number of problems like low bioavailability, first pass metabolism and GIT degradation. To overcome the above mention problem Lodzki et al. prepared CBD-ethosomal formulation for transdermal delivery. Results of the skin deposition study showed significant accumulation of CBD in skin and underlying muscles after application of CBD-ethosomal formulation to the abdomen of ICR mice Plasma concentration study showed that steady state level was reached in 24 hr and maintained through 72 hr. Significantly increased in biological anti-inflammatory activity of CBD-ethosomal formulation was observed when tested by carrageenan induced rat paw edema model. Finally, it was concluded that encapsulation of CBD in ethosomes significantly increased its skin permeation, accumulation and hence its biological activity.[9,10]

DELIVERY OFANTIBIOTICS

Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of these agents. Conventional oral therapy causes several allergic reactions along with several side effects. Conventional external preparations possess low permeability to deep skin layers and subdermal tissues. Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotic into deeper layers of skin. Ethosomes penetrate rapidly through the epidermis and bring appreciable amount of drugs into the deeper layer of skin and suppress infection at their root. With this purpose in mind Godin and Touitou prepared bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery.[10]

Experiments revealed that ethosomes facilitated the co-penetration of antibiotic and phospholipid into cultured 3T3 Swiss albino mice fibroblasts. The data obtained by CLSM experiment was confirmed by FACS techniques and it was found that ethosomes penetrated the cellular membrane and released the entrapped drug molecules within the cells. The results of this study showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy.[9]

DELIVERY OF ANTI-VIRAL DRUGS

Zidovudine is a potent antiviral agent acting on acquired immunodeficiency virus. Oral administration of zidovudine is associated with strong side effects. Therefore, an adequate zero order delivery of zidovudine is desired to maintain expected anti-AIDS effect.[112, 113] In a recent study the optimized ethosomal formulation exhibited a transdermal flux of 78.52.5 g/cm2/h across rat skin, while the hydroethanolic solution gave a flux of only 5.20.5 g/cm2/h of zidovudine. The flux from ethanolic solution was found to be 7.2-0.6 g/cm2/h. Jain et al. concluded from this study that ethosomes could increase the transdermal flux, prolong the release and present an attractive route for sustained delivery of zidovudine.[10]

Acyclovir is another anti-viral drug that widely used topically for treatment of Herpes Labialis. The conventional marketed acyclovir external formulation is associated with poor skin penetration of hydrophilic acyclovir to dermal layer resulting in weak therapeutic efficiency. It is reported that the replication of virus takes place at the basal dermis. To overcome the problem associated with conventional topical preparation of acyclovir, Horwitz et al. formulated the acyclovir ethosomal formulation for dermal delivery. They have clinically evaluated its performance in a double blind, randomized study with marketed formulation of acyclovir (Zovirax, Glaxo-Wellcome) in terms of time to crust formation, time to loss of crust and proportions of lesions not progressive beyond the popular stage (abortive lesions).

Significant improvement in all evaluated clinical parameters was observed when disorder was treated with ethosomal formulation in comparison to marketed formulation. The average time to crusting of lesions was 1.6 vs 4.3 days in the parallel arm and 1.8 vs. 3.5 days in the crossover arm ($P<0.025$) for ethosomal acyclovir and Zovirax, respectively. Hence, shorter healing time and higher percentage of abortive lesions were observed when acyclovir was loaded into ethosomes.[10]

DELIVERY OF PROBLEMATIC DRUG MOLECULES

The oral delivery of large biogenic molecules such as Peptides or Proteins is difficult because they are completely degraded in the GI tract. Non-invasive delivery of proteins is a better option for overcoming the problems associated with oral delivery. Dkeidek and Touitou investigated the effect of ethosomal insulin delivery in lowering blood glucose levels (BGL) in vivo in normal and diabetic rats. In this study a Hill Top patch containing insulin Ethosomes wasapplied on the abdominal area of an overnight fated rat. The result showed that insulin delivered from this patch produced a significant decrease (up to 60%) in BGL in both normal and diabetic rats. On the other hand, insulin application from a control formulation was not able to reduce the BGL.[9,10,11]

Verma and Fahr[80] reported the cyclosporin A ethosomal formulation for the treatment of inflammatory skin disease like psoriasis, atopic dermatitis and disease of hair follicle like alopecia areata etc. They have combined the ethanol with a commercially lipid mixture NAT 8539 contained Phosphatidylcholine (73-75%), lyso-phosphatidylcholine (upto 6%), Cephaline (upto 4%) and Phosphatidic acid (upto 6%) and natural oils. They have found that cyclosporine vesicles prepared with NAT 8539/ethanol (10/3.3) showed 2.1 fold, NAT 8539/ethanol (10/10) showed a 4.4 fold and NAT 8539/ethanol (10/20) showed a 2.2 higher deposition of cyclosporine into SC as compared to vesicle made of NAT 8539 without ethanol. As the concentration of ethanol increased the depth and intensity of fluorescence was increased. Formulation NAT 8539/ethanol ((10/10) produced a fairly homogeneous bright fluorescence throughout the SC. They have concluded that ethanolic liposomal formulation can be used for the topical delivery of problematic drug molecules like cyclosporine whose oral delivery is difficult.[10,11]

Paolino et al. investigated the potential application of ethosomes for dermal delivery of ammonium glycyrrhizinate. Ammonium glycyrrhizinate is naturally occurring triterpenes obtained from Glycyrrhizinate Glabra and useful for the treatment of various inflammatory based skin diseases. In vitro skin permeation experiments showed the significantly ($P<0.001$) higher cumulative amount of drug permeated from ethosomes (63.2%) than hydroalcoholic solution (22.3%) and aqueous solution (8.9%) of ammonium glycyrrhizinate. They have also evaluated the human skin tolerability using Reflectance Spectrophotometry that is a non-invasive technique to evaluate the carrier toxicity. Ethosomal formulation showed a very good skin tolerability in human volunteer even applied for 48 hr. Biological anti-edema

activity also showed the significant enhanced in case of ethosomal formulation as compared to ethanolic or aqueous solution of drug.[10,11]

NEED FOR THE STUDY

Enalapril maleate is an ACE inhibitor. It is used for the treatment of hypertension.

Enalapril maleate is poorly absorbed following an oral dose. Major side effects are hypotension, taste disturbance, diarrhoea, nausea, vomiting. The minimum dose of Enalapril maleate is 5 mg/day.[10]

An alternative approach to overcome the low oral bioavailability is to administer the drug by non oral routes such as buccal, nasal, vaginal, transdermal and parenteral. Among the above routes the transdermal delivery of ethosome is advantageous. Because it has good penetrability, ease of administration, rapid terminatin of the therapy and administratin to unconscious patients.[11]

Ethosome mainly contain phospholipids with higher concentration of ethanol. It can beused for systemic delivery of drug. It is beneficial in case of Enalapril maleate to overcome theproblem of frequent dosing due to its shorter half-life. Prolonged release of the drug and increased bioavailability leads to significant reduction in the dose and hence dose related side effects.

In the present investigation, an attempt will be made to formulate Enalapril maleateethosomes in order to increase bioavailability and reduce side effects.[11]

VESICULAR CARRIERS FOR TOPICAL DELIVERY OTHER THAN ETHOSOMES

In the early 1990s, a greater knowledge of vesicles were gained and many types of vesicles and vesicles derivatives have been tested for their abilities for transdermal delivery of drug. Most experiments however have centered on liposomes, since derivatives only add to their basic properties. Vesicles are closed, spherical membranes that separate a solvent core from the surrounding solvent. They are typically composed of phospholipids, mainly phosphotidyl choline (PL) as in liposomes while it has been suggested that the external envelop of a liposome would allow it to pass through lipophilic skin. Most researches show that liposomal vesicles become trapped within the top layer of the stratum corneum cells.[10,11]

Darr D Dunston et al., 21 (1996) demonstrated that Vitamin E liposomes provide time release, target delivery of essential antioxidant power of superior benefits. Use morning and evening for deep hydration of your skin that will substantially diminish the visible signs of premature aging and provide a refreshing glow and youthful resilience, visible results in 14 days.

Arsi and Vuleta G., 22 (1999) prepared Liposomes incorporated with Vitamin A, in polyacrylate hydrogel. Liposomes are made of purified lecithin, which has higher phospholipid.[11]

Content and exerts better anti-oxidative properties. The relatively poor stability of Vitamin A encapsulation in the liposomes made from the purified phospholipid fraction (90% Phosphatidyl choline). It increases the Vitamin A stability during UV radiation in pure liposome dispersion or liposomes with Vitamin A incorporated in polyacrylate gel as vehicle.[11]

Khandare J N et al., (2001) have done the preparation and evaluation of Nimesulide niosomes for topical application. Nimesulide was encapsulated into niosomes using five different surfactants (Tween 80 and 60, Span 80, 60 and 20) in different ratios by ether injection technique. The preparations were studied for the encapsulation efficiency and in vitro drug release. These niosomal preparations were evaluated for anti inflammatory activity after incorporating them into a gel base.

Satturwar P M et al., 25 (2001) carried out the work of niosomal delivery of ketoconazole, antifungal drug. Ketoconazole was encapsulated in niosomes for topical application.

Ketoconazole niosomes were prepared by thin film hydration technique using surfactant (Tween 40 or 80), cholesterol and drug in five different ratio (by weight). The prepared niosomes were characterized for size, shape, entrapment efficiency and in vitro drug release (by exhaustive dialysis). Niosomes were then formulated and tested for in vitro antifungal activity (Cup plate method).[11,12]

Amit B et al., 26 (2004) designed and characterized topical liposomes using Tamoxifen, were prepared by thin film hydration method. Liposomal formulation of Tamoxifen were evaluated for in vitro skin permeation, using mice skin and results were compared with that of aqueous solution and carbopol gel containing Tamoxifen in equal amounts. The size of multilamellar

liposomes were found in range of 1 to 13 m and the maximum loading of tamoxifen was noted to be 57.5%. Liposomes stored at 2 to 80C were found to be most stable with only 5% drug loss over the storage period of 5 weeks. Significantly higher skin permeation of Tamoxifen from liposomal formulations has been achieved as compared to solution and carbopol gel containing Tamoxifen. Higher magnitude of Tamoxifen retention in the skin layer was noted with liposomal formulations than non- liposomal formulations of the drug.[12]

Chetoni P et al., 27 (2004) investigated liposomal formulation for topical administration of acyclovir in comparison with a commercial acyclovir ointment, by determining the pharmacokinetic profile of the drug in the aqueous humor of rabbits after topical administration. The acyclovir liposomal dispersion produced a significantly higher drug concentration profile in aqueous with respect to other reference formulation and containing the same acyclovir concentration and showed a 90 min plateau. In spite of the much higher dose (1.5 versus 0.18 mg), the AUC produced by full strength 3% ointment was only 1.6 times greater than that corresponding to liposomal vesicle.[11]

NOVEL VESICULAR CARRIER- ETHOSOMES

Classic liposomes are of little or no value as carriers for transdermal delivery because they do not deeply penetrate the upper layer of stratum corneum. Only specially designed vesicles were shown to be able to allow transdermal delivery. Ethanol is known as an efficient permeation enhancer. Touitou et al., 2000 discovered lipid vesicular system embodying ethanolin relatively high concentration, which was named ethosomes. The ethosomes penetrate skin and enhance compound delivery to deep skin strata or systemically; Touitou et al., 2000, Dayan and Touitou., 2000. Touitou et al., 2000 suggested that ethanol fluidizes both ethosomal lipids and bilayers of the mortar. The soft malleable vesicles then penetrate through the disorganized lipid bilayers.[11,12,13]

Horwitz E et al.,14 (1999) evaluated the efficiency of 5% Acyclovir in a novel liposomalcarrier (ethosomes) in comparison with that of a commercial 5% Acyclovir cream (Zovirax cream) and that of drug free vehicle in the treatment of recurrent herpes labialis in a 2-armed, double-blind randomized clinical study and found the time to crusting with the ethosomal acyclovir (1.6 day) was significantly shorter than the times with the acyclovir cream (4.3 days) and the time with the drug free vehicle (4.8 days) in this arm, the shorter time to loss of crust forthe ethosome (3.5 days), in comparison with the times for the cream (6.4 days) and the drug freevehicle (6.1 days), did not reach statistical significance.

Touitou E et al.(1999) designed and evaluated novel vesicular carrier Ethosomes of testosterone, molecular probes and Minoxidil for characterization and skin penetration properties. Testosterone, molecular probes and Minoxidil were formulated as Ethosomes using Soyabean phosphatidyl choline. The size of the vesicles increased with decreasing ethanol concentration. Skin permeation study was carried out with Rhodamine red(RR) which show significant greater penetrability from ethosomal preparation while penetration from liposomes was negligible.[11]

Nava Dayan et al.(2000) characterized a novel Ethosomal carrier containing trihexyphenidyl HCl (THP) and to investigate the delivery of THP from Ethosomes versus classic Liposomes.

As the THP concentration was increased from 0 to 3%, the size of the vesicles decreased from 154 to 90 nm and zeta potential value increased from –4.5 to +10.4. In contrast, THP liposomes were much larger and their charge was not affected by THP. When compared with standard liposomes, Ethosomes had a higher entrapment fluorescent probe to thedeeper layer of skin. The concentration of THP in Ethosomes was 4.5 times higher that from liposomes, phosphate buffer and hydroethanolic solution.

Jain S et al.,(2003) Designed and evaluated novel vesicular carrier ethosomes of zidovudine for enhanced transdermal delivery. Zidovudine was formulated as Ethosomes using Soya phosphatidyl choline (PC) as phospholipid. Ethosomes containing 10%, 20%, 30%, 40% and 50% ethanol, 0.4% drug and 2% PC were prepared. Ethosomes containing 30% ethanol showed highest drug entrapment. The size of the Ethosomes varies from 91 to 35nm. The quantitative determination was performed by HPLC. The vesicles size and size distribution were determined by DLS. The in vitro release studies of ethosomes and liposomes were carried out at 370C for 24 hours using locally fabricated diffusion cell. The release of the drug from ethosomes containing 30 % ethanol was found to be highest than otherformulation of ethosomes, liposomes, 30 % hydro-alcoholic solution and an ethanolic phospholipids solution.[11,12,13]

Lodzki M et al., 13 (2003) designed a transdermal delivery system for Cannabidiol (CBD) by using ethosomal carrier. CBD ethosomes were characterized by transmission electron microscopy, confocal laser scanning microscopy and DSC. In vivo application of ethosomal CBD to nude mice produce a significant accumulation of the drug in the skin and in underliningmuscle. Upon transdermal application of the ethosomal system to the abdomen of

ICR mice for 72 hour. Transdermal application of ethosomal CBD prevented the inflammation and edema induced by sub-plantar injection of carrageenan in the same animal model.

Godin B et al. (2004) designed and characterized mechanism of Bacitracin permeation enhancement through the skin and cellular membranes from an Ethosomal carrier. The main objective of present work was to investigate the dermal and intracellular delivery of Bacitracinfrom Ethosomes.[13]

Bacitracin and fluorescently labeled bacitracin (**FITC-Bac**) ethosomes were characterized for shape, lamellarity, fluidity, size distribution and entrapment capacity by scanning electron microscopy (SEM), transmission electron microscopy (TEM), differential scanning calorimetry (DSC), dynamic light scattering (DLS) and ultracentrifugation, respectively. Confocal laser scanning microscopy (CLSM) experiments revealed that ethosomesfacilitated the co-penetration of antibiotic and phospholipid into swiss albino mice fibroblasts.

Fluorescent-activated cell sorting (FACS) experiments suggested that ethosomes penetrate cellular membrane releasing the entrapped molecule within cells. FITC-Bac from ethosomal system in in vitro and in vivo experiments, demonstrated that the antibiotic peptide was delivered into deep skin layer.[11]

ADVANTAGE OF ETHOSOMES OVER ALL OTHER TRANSDERMAL DRUG DELIVERY SYSTEMS

To improve the permeation of drugs through the skin various mechanisms have been investigated, including use of chemical or physical enhancers, such as Iontophoresis, Sonophoresis, etc. Liposomes, Niosomes, Transferosomes and ethosomes also have been reportedto enhance permeability of drug through the stratum corneum barrier.

Permeation enhancers increase the permeability of the skin, so that the drugs can cross through.

The skin easily. Unlike classic liposomes, that are known mainly to deliver drugs to the outer layers of skin, ethosomes can enhance permeation through the stratum corneum barrier. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic.[11,12]

The ethosomes more advantages when compared to transdermal and dermal delivery. It

delivers large molecules such as peptides, protein molecules. Simple method for drug delivery incomparison to Iontophoresis and Phonophoresis and other complicated methods.

Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature. High patient compliance as it is administrated in semisolid form (gel or cream) and various application in Pharmaceutical, Veterinary, Cosmetic field.[12,13]

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 controlover 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 thatethosomal formulations possess promising future in effective dermal/transdermal delivery of bioactive agents.[11,12,13]

CONCLUSION

Transdermal route is promising alternative to drug delivery for systemic effect. An attempt was made to formulate the highly efficient ethosomal drug delivery system and Enalapril meleate is used as model drug. The method described by Touitou et al., (2000) was employed for the preparation of various ethosomal formulation containing different concentration of ethanol (20%to 40%) with sonication and without sonication. Liposomal formulation was also prepared by the thin film hydration method. The techniques used were simple and reproducible. The prepared Ethosomes were spherical and discrete in shape. The size of vesicles were found to be in the range of 3.26-5.79 μm,0.716-1.301 μm and 5.32 μm for unsonicated ethosomes, sonicated ethosomes and liposomes respectively. However ethosomes prepared by sonication method weremore uniform and smaller in size, which is essential for skin permeation. While comparing theentrapment efficiency, ethosomes containing 30% w/w ethanol and prepared by sonication showed highest value with respect to all other formulation, so it is concluded ethosomes prepared by sonication and containing 30%

w/w ethanol as the best formulation considering all other aspects. The highest value of transdermal flux for sonicated ethosomes containing 30% w/w ethanol is the indication of complete and rapid penetration through the skin may be because of tiny vesicular size. This is an encouraging observation for drugs, which are poorly absorbed fromskin.

All the formulation of ethosomes showed a zero order release for in-vitro release studies. Though the ethosomes are rapidly penetrated through the skin, there is variation between the sonicated and unsonicated products. Stability studies carried out for a period of 8 weeks showed no changes in the charecterisation of ethosomes and further the loss of drug is not more than 3%. When effect of sonication was compared on ethosomal formulation, sonicated formulations are possessed better or suitable characterization (smaller size, uniform size distribution, highest entrapment efficiency and higher transdermal flux) as compared with unsonicated formulation. From the above observation it can be concluded sonication is essential tool for the preparation of ethosomes. An extensive investigation is needed with reference to depth of penetration into the skin, determination of zeta potential and confirmation of configuration of phospholipid in lipid bilayer. There is a need to develop suitable transdermal formulation by using prepared ethosomes for transdermal application and for commercial exploitation. Thus, the specific objective listed in the introduction chapter of this thesis were achieved namely design, characterization and release studies of enalapril maleate ethosomes. Certainly these finding can be applied for transdermal drug delivery of enalapril maleate for treatment of hypertension. Further, these finding may help the industry for development and scaling up a new formulation.[12,13]

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