ETHOSOMES: A NOVEL APPROACH TO DRUG DELIVERY SYSTEM

[1]Juhi Namdev*, [2]Aman Pandey, [3]Sarvesh Gupta, [4]Shubham kumar

[1]Department of Pharmacy, Lakshmi Narain College Of Pharmacy, Bhopal, India.

[2]Department of Pharmacy, Patel Group Of Colleges, Bhopal, India.

[3]Department of Pharmacy, Truba Institute Of Pharmacy, Bhopal, India.

[4]Department of Pharmacy, Lakshmi Narain College Of Pharmacy, Bhopal, India.

Abstract : Transdermal Drug Delivery System is a self contained, discrete dosage forms patches applied to the intact skin for the delivery of drug through the skin at a controlled rate to the systemic circulation. transdermal patch is a medicated adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and into the bloodstream and this promotes healing to an injured area of the body. skin the major organ of the body that serves as a potential route of drug delivery for local and systemic effects. the outermost layer of skin, the stratum corneum acts as a active barrier that prevents penetration of hydrophilic and high molecular weight drugs. Ethosomes are a novel phospholipid vesicular carrier containing high ethanol concentrations and offer improved skin permeability and efficient bioavailability due to their structure and composition. This article gives a review of ethosomes including anatomy of skin, absorption of ethosomes, compositions, advantages, disadvantages, classification, herbal formulations, influence of high alcohol content, method of preparation, mechanism of action, therapeutic applications, evaluation parameters, safety studies, future prospects and marketed products of ethosomes.

IndexTerms **- Ethosomes, noninvasive, transdermal drug delivery, vesicular carriers, skin Permeation, therapeutic effect.**

INTRODUCTION

Transdermal drug delivery system (TDDS) showed promising result in comparison to oral drug delivery system as it eliminates gastrointestinal interferences and first pass metabolism of the drug but the main drawback of TDDS is it encounters the barrier properties of the Stratum Corneum i.e. only the lipophilic drugs having molecular weight < 500 Dalton can pass through it[1,2]. 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 reported to 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. They are known mainly to deliver drugs to the outer layers of skin, ethosomes can enhance permeation through the stratum corneum barrier. [34,37]

ETHOSOMES

Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol or isopropyl alcohol) in highly relative concentration in water. They are noninvasive delivery carriers that enable drugs to reach deep into the skin layers and/or the systemic circulation. Ethosomes are the slight modification of well established drug carrier liposome. These are soft, malleable vesicles tailored for enhanced delivery of active agents.

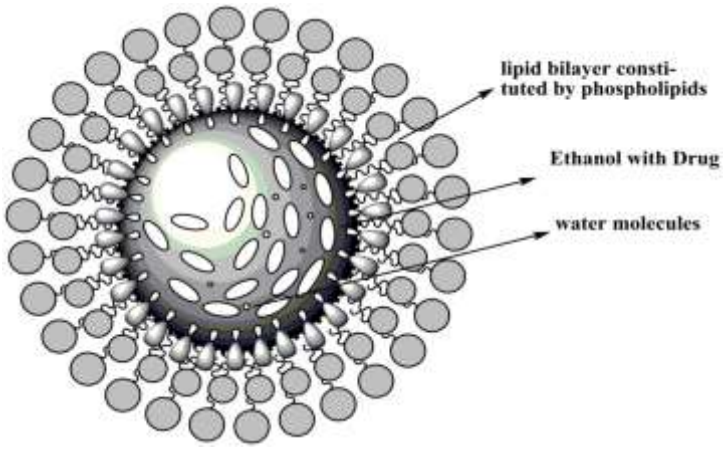


Fig.1: Strucuture of ethosomes

Vesicles would also allow controlling the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and thus be able to release just the right amount of drug and keep that concentration constant for longer periods of time. The size range of ethosomes may vary from tens of nanometers (nm) to microns (μ)[37]. ethosomes permeate through the skin layers more rapidly and thus possess significantly higher transdermal flux[11].

ADVANTAGES OF ETHOSOMES

1. Delivery of large molecules (peptides, protein molecules) is possible.
2. It contains non-toxic raw material in formulation.
3. Enhanced permeation of drug through skin for transdermal drug delivery.
4. The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
5. They are the advanced and most effectively novel delivery system in the body system[32,34,44].

DISADVANTAGES OF ETHOSOMES

1. Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it usually designed to offer slow, sustained drug delivery.
2. Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
3. The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
4. Adhesive may not adhere well to all types of skin.
5. The main advantage of ethosomes over liposomes is the increased permeation of the drug[4,9,10].

ANATOMY OF SKIN

Human skin is an effective, selective barrier to chemical permeation, although the skin as a route for delivery can offer many advantages, including avoidance of first-pass metabolism, lower fluctuations in plasma drug levels, targeting of the active ingredient for a local effect, and good patient compliance. Water soluble molecules and drugs are normally not able to cross the skin as the skin is a natural barrier to water. The stratum corneum is composed of insoluble bundled keratins surrounded by a cell envelope, stabilized by cross-linked proteins and covalently bound lipids. the epidermis (specifically the stratum corneum) provides the major control element; most small, water-soluble, and non-electrolytes diffuse into the systemic circulation a thousand times more rapidly when the horny layer is present. Thus, to maximize the flux of the drug, the barrier hindrance is reduced by various approaches. Several technological advances have been made in the recent decades to overcome skin barrier properties. Examples include physical means such as iontophoresis, sonophoresis, microneedles, and chemical means, using penetration enhancers and biochemical means, such as, liposomal vesicles and enzyme inhibition. The physical means like iontophoresis, microneedles, and sonophoresis are relatively complicated to use, and will affect patient compliance.

The lipids present in the skin contribute to the barrier properties of the skin and prevent the systemic absorption of drugs. Due to the amphiphilic nature, lipid vesicles may serve as non-toxic penetration enhancers for drugs. In addition, the vesicles can be used for encapsulating hydrophilic and lipophilic as well as low and high molecular weight drugs. [32-38]

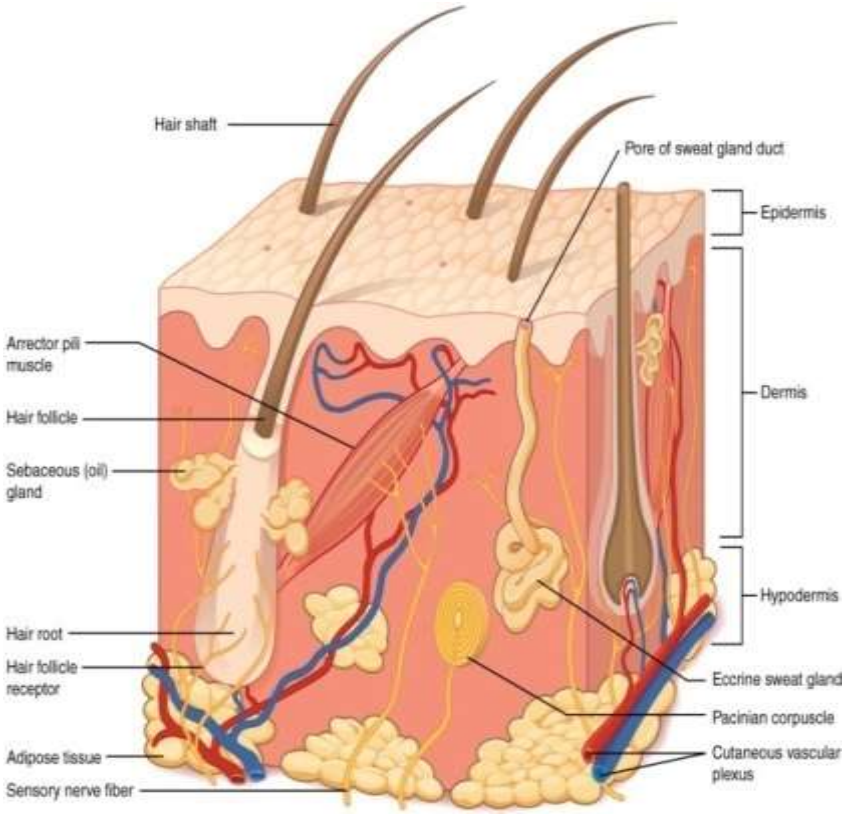


Fig.2: Anatomy of skin.

FUNCTIONS OF SKIN

1. They Provides a protective barrier against mechanical, thermal and physical injury and no noxious agents.
2. Prevents loss of moisture.
3. Reduces the harmful effects of UV radiation.
4. Acts as a sensory organ.
5. Helps regulate temperature control.
6. Plays a role in immunological surveillance.
7. Synthesizes vitamin D3 (cholecalciferol) [7-12].

ABSORPTION OF ETHOSOMES

The skin is the largest organ of the body. The skin an average adult body is about 20 square feet and it received about one third of total available blood. The skin is multilayered organ composed of three histological tissues The outermost layer of skin, epidermis is which provides a waterproof barrier and creates our skin tone. Dermis, beneath epidermis, contains tough connective tissue, hair follicles, and sweat glands and deeper subcutaneous tissue (hypodermis) is made of fat and connective tissue.

Human skin consists of a stratified, cellular epidermis and an underlying dermis of connective tissue; Epidermis and Dermis.

1. Epidermis

The four layers of the epidermis are:

1. Stratum basale (basal or germinativum cell layer)
2. Stratum spinosum (spinous or prickle cell layer)
3. Stratum granulosum (granular cell layer)
4. Stratum corneum (horny layer).

2. **Dermis**

The dermis varies in thickness, ranging from 0.6 mm on the eyelids to 3 mm on the back, palms and soles. It is found below the epidermis and is composed of a tough, supportive cell matrix. Two layers comprise the dermis: A thin papillary layer and a thicker reticular layer.

3. **Percutaneous Absorption**

Percutaneous absorption involves passive diffusion of substances through the skin. The mechanism of permeation can involve passage through the epidermis itself or diffusion through shunts, particularly those offered by the relatively widely distributed hair follicles and exocrine glands.

4. Transepidermal absorption

The trans-epidermal pathway is principally responsible for diffusion across the skin. The main resistance encountered along this pathway arises in the stratum corneum. Permeation by the trans-epidermal route first involves partitioning into the stratum corneum. Diffusion then takes place across this tissue. The current popular belief is that most substances diffuse across the stratum corneum via the intercellular lipoidal route. However, there appears to be another microscopic path

through the stratum corneum for extremely polar compounds and ions. When a permeating drug exits at the stratum corneum, it enters the wet cell mass of the epidermis and since the epidermis has no direct blood supply, the drug is forced to diffuse across it to reach the vasculature immediately beneath. It is a permeable field that functions as a viscid watery regime to most penetrants. It appears that only ions and polar non-found at the hydrophilic extreme and lipophilic non-electrolytes at the hydrophobic extreme have any real difficulty in passing through the viable field. The epidermal cell membranes are tightly joined and there is little to no intercellular space for ions and polar non-electrolyte molecules to diffusionally squeeze through. Passage through the dermal region represents a final hurdle to systemic entry. Permeation through the dermis is through the interlocking channels of the ground substance. Since the viable epidermis and dermis lack major physicochemical distinction, they are generally considered as a single field of diffusion, except when penetrants of extreme polarity are involved, as the epidermis offers measurable resistance to such species.

5. Transfollicular (shunt pathway) absorption

The skin's appendages offer only secondary avenues for permeations. Sebaceous and eccrine glands are the only appendages which are seriously considered as shunts.By passing the stratum corneum since these are distributed over the entire body. Though eccrine glands are numerous, their orifices are tiny and add up to a miniscule fraction of the body's surface. Moreover, they are either evacuated or so profusely active that molecules cannot diffuse inwardly against the gland's output. For these reasons, they are not considered as a serious route for percutaneous absorption. However, the follicular route remains an important avenue for percutaneous absorption since the opening of the follicular pore, where the hair shaft exits the skin, is relatively large and sebum aids in diffusion of penetrants. Partitioning into sebum, followed by diffusion through the sebum to the depths of the epidermis, mechanism permeation by this route. Vasculature subserving the hair follicle located in the dermis is the likely point of systemic entry.

6. Clearance by local circulation

The earliest possible point of entry of drugs and chemicals into the systemic circulation is within the papillary plexus in the upper dermis. The process of percutaneous absorption is general, regarded as ending at this point. However, some molecules bypass the circulation and diffuse deeper in the dermis[8-43].

CLASSIFICATION OF ETHOSOMES

Ethosomes are classified mainly into the three categories on the basis of their characteristic properties. Therefore they are classical, binary and trans ethosomes.

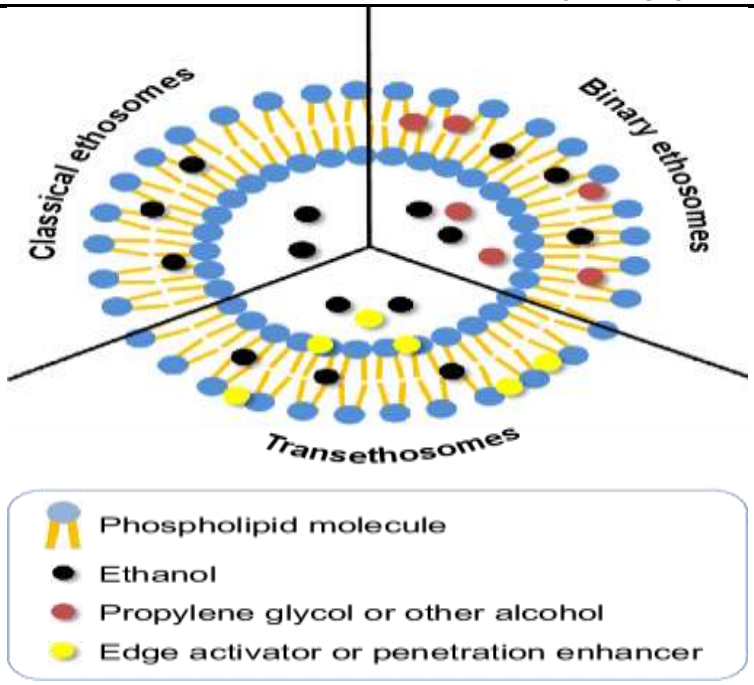


Fig.3: ethosomes classification on the bsasis of actions.

S.No.	Parameters	Classical Ethosomes	Binary Ethosomes	Trans Ethosomes
1	Composition	- phospholipids - ethanol - stabilizer - water - charge inducer - drug / agent	- phospholipids - ethanol - propylene glycol or other alcohol - water - charge inducer - drug / agent	- phospholipids - ethanol -edge activator (surfactant) or penetration enhancer - water - charge inducer - drug / agent
2	Morphology	spherical	spherical	Regular, irregular or spherical
3	Size	Smaller than liposomes	Equal or smaller than classical ethosomes	Size based on the type of concentration of penetration enhancer used
4	Potential	Negatively charged	Negatively charged	Positively or Negatively charged
5	Entrapment efficiency	Higher than liposomes	Typically higher than classical ethosomes	Typically higher than classical ethosomes
6	Skin permeation	Typically higher than classical liposomes	Typically higher or equal than classical ethosomes	Typically higher than classical ethosomes
7	stability	Stable than classical liposomes	Stable than classical ethosomes	Not particularly determined

Table no.1: classification of ethosomes

COMPOSITION OF ETHOSOMES [34]

Ethosomes are composed mainly of phospholipids, (phosphatidylcholine, phosph-atidylserine, phosphatitidic acid), high concentration of ethanol and water. The non-aqueous phase range between 22 % to 70 %. The alcohol may be ethanol or isopropyl alcohol. The high concentration of ethanol makes the ethosomes unique, as ethanol is known for its disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. 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 and improves drug distribution ability in stratum corneum lipids[8-11].

S.No.	Class	Example	Uses
1.	Phospholipid	Soya phhosphatidyl choline Dipalmityl phosphatidyl choline Egg phosphatidyl choline Distearyl phosphatidyl choline	Vesicles component
2.	Polyglycol	Propylene glycol Transcutol RTM	Skin penetration enhancer
3.	Alcohol	Ethanol Isopropyl alcohol	To provide softness for vesicle membrane and penetration enhancer
4.	Cholestrol	Cholestrol	To provide stability to vesicle membrane
5.	Dye	Rhodamine-123 Rhodamine red Fluorescence isothiocynate (FITC)	For characterization studies
6.	Vechile	Carbopol 934	As a gel former

Table no.2: The different types of additives involve in composition or formulation of ethosomes.

INFLUENCEMENT HIGH ALCOHOL CONTENT IN ETHOSOMES

Ethanol is an established efficient permeation enhancer and is present in quite high concentration (20-50%) in ethosomes. However, due to the inter digitation effect of ethanol on lipid bilayers, it was commonly believed that vesicles could not coexist with high concentration of ethanol. Touitou discovered and investigated lipid vesicular systems embodying ethanol in relatively high concentration and named them ethosomes. The basic difference between liposomes and combination of relatively high concentration of ethanol (20-50%) in vesicular form in ethosomes was suggested to be the main reason for their better skin permeation ability. The high concentration of ethanol (20-50%) in ethosomal formulation could disturb the skin lipid bilayer organization. Therefore, when integrated into a vesicle membrane, it could give an ability to the vesicles to penetrate the SC. Furthermore, due to high ethanol concentration the ethosomal lipid membrane was packed less tightly than conventional vesicles but possessed equivalent stability. This allowed a softer and malleable structure giving more freedom and stability to its membrane, which could squeeze through small openings created in the disturbed SC lipids. In addition, the vesicular nature ethosomal formulations could be modified by varying the ratio of components and chemical structure of the phospholipids. The versatility of ethosomes for systemic delivery is evident from the reports of enhanced delivery of quite a few drugs like acyclovir, minoxidil, triphexyphenidyl, testosterone, cannabidol and zidovudine.

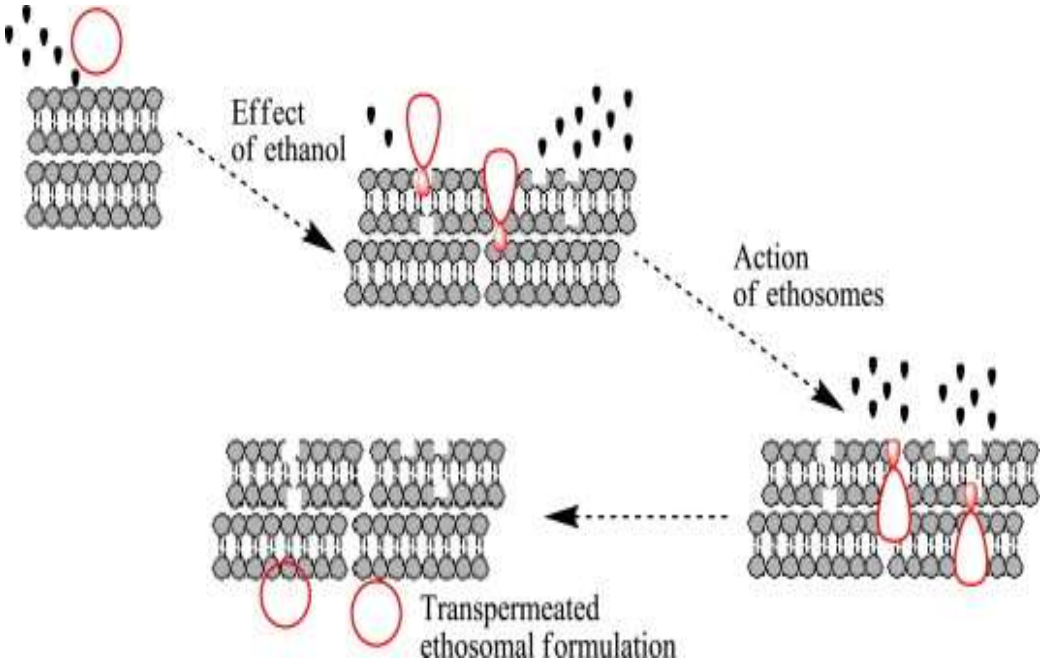


Fig.4: Effect high alcohol content on ethosomes.

SOME FORMULATIONS OF HERBAL ETHOSOMES

There are some herbal drug or herbal constituent containing a effective action by formulating in an appropiate ethosomal preparation which were used to treat the below given diseases and disorders. Therefore, they are novel herbal ethosomal formulation which is highly potent in the pharmacological of the drug on an specific area or for and treatment of a disease.

S.No.	Botanical Name	Formulation	Active Ingredients	Biological Activity	Application	Method of Preparation
1	Podophyllum hexandrum	podopyllotoxin	Etoposide & teniposide	Purgative, anti-tumor, anti-viral, anti-rheumatic	Higher entrapment efficiency and enhance therpaeutic effect	Solvent dispersion method
2	Glycyrrhiza glabra	Ammonium glycyrrhizinate ethosomes	Glycrrhizic acid	Anti-inflammatory	Increases of ivitro percutaneous permeation and significantly enhanced anti-inflammatory activity	Solvent dispersion method
3	Triptergium wilfordi	tripolide	Diterpene triepoxide	Anti-inflammatory	High entrapment efficiency and good percutaneous permeability	Combining filming rehydration method and ultrasonic method
4	Sophora alopecuroides	Sophora ethosome	Sophocarpine, matrine, oxymatrine, sophordine	Anti-cancer, Anti-endotoxic. Anti-inflammatory	Ehance drug delivery and stability	Transmembrane pH gradient active loading method
5	Sesbania grandiflora	Sesbania ethosome	Leucocyanidin, cyanidin	Anti-microbial	Enhance transdermal permeation	Solvent dispersion method
6	Sophora flavesens	Matrine ethosome	Matrine, oxymatrine alkaliods	Cardioprotective, Anti-inflammatory	Improve percutaneous permeation	Solvent dispersion method

Table no.3: Herbal formulations of ethosomes with their properties and actions.

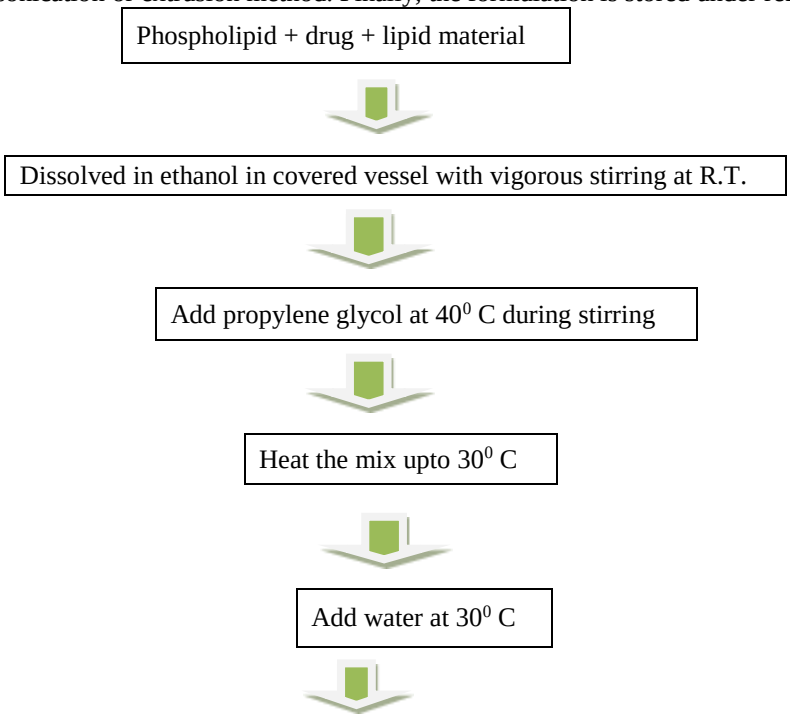
METHOD OF PREPARATION OF ETHOSOMES[40-45]

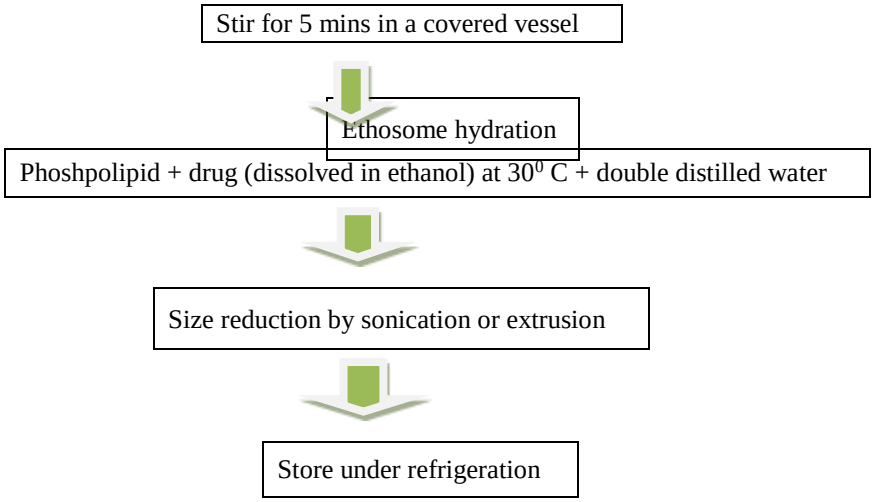
There are four methods which can be used for the formulation and preparation of ethosomes. The following methods are as follows:

1.Cold method
2. Hot method
3. Classic method
4. Mechanical dispersion method

1. Cold Method

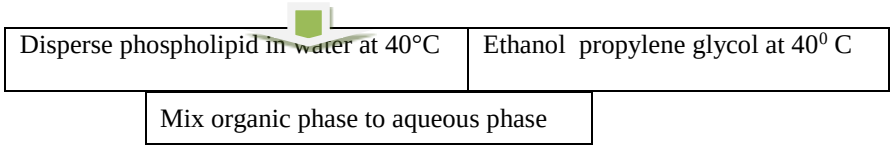
This method is most commonly used for the preparation of ethosomal formulation. In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of the mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 30ºC in a water bath. The water heated to 30ºC in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of an ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation is stored under refrigeration[13-19]





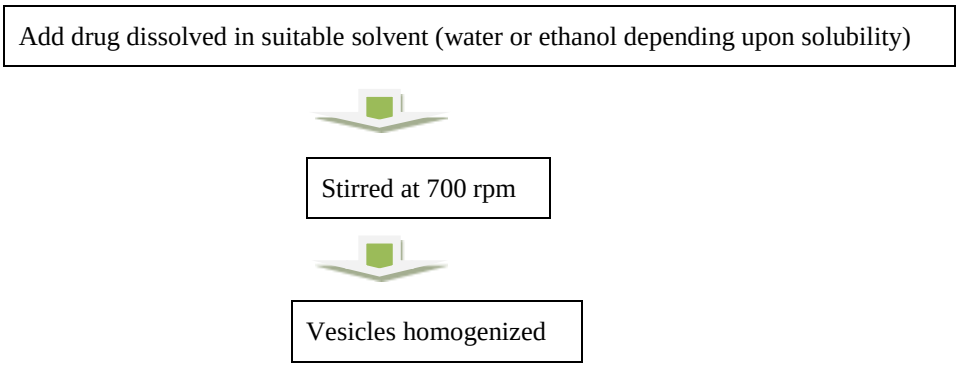
2. Hot method

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



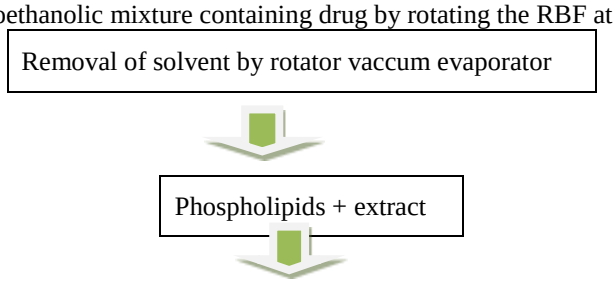
3. Classic method

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

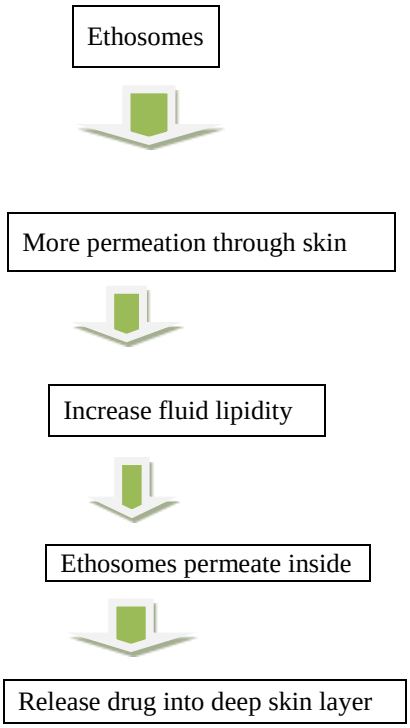


4. Mechanical dispersion method

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



MECHANISM OF ACTION OF ETHOSOMES



MECHANISM OF DRUG PERMEATION IN ETHOSOMES[17-32]

This can be summarized into main pathways:

1. Ethanol effect
2. Ethosomal effect

1. Ethanol effect

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

2. Ethosomal effect

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

THERAPEUTIC APPLICATIONS OF ETHOSOMES[30-44]

The following are the applications of ethosomes or ethosomal drug delivery system are given below:

1. **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-CMVdriven transfecting construct into ethosomal formulation. They applied this formulation to the dorsal skin of 5-week male CD1 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[49-51].

2. 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 hydroethanolicsolution, 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.

3. Transcellular delivery

Touitou et al. M from ethosomes, hydroethanolic solution and liposomes, respectively. Maximum fluorescence intensities measured for RR delivered from ethosomes, hydroethanolic solution and liposomes were 150, 40 and 20 arbitrary units (AU), respectively. Fibroblasts viability tests showed that the ethosomal carrier was not toxic to the cultured cells. investigated the efficiency of transcellular delivery of ethosomes in Swiss albino mice 3T3 fibroblast. The probes chosen for study were D-289[4(4(diethylamino)styrylNmethylpyridinumiodide],rhodaminered[dihexadecanoylglycerophosphoethanolamine] and fluorescent phosphatidylcholine. The penetration of these fluorescent probes into fibroblasts and nude mice skin was examined by CLSM (Confocal Laser Scanning Microscopy) and FACS (Fluorescent Activated Cell Sorting) techniques. CLSM micrograph showed that significant quantity of probe was penetrated into the cells when incorporated into ethosomes as evident from the high intensity of fluorescence. In comparison, incorporation into hydroethanolic solution or classic liposomes produced almost negligible fluorescence. The intracellular presence of each of the three probes tested was evident after 3 min. of incubation. Enhanced delivery of the hydrophilic calcein and lipophilic rhodamine red (RR) probe to nude mice skin was also observed when incorporated into ethosomes. Calcein penetrated the skin to a depth of 160, 80 and 60 Touitou et al. in their further study demonstrated better intracellular uptake of bacitracin, DNA and erythromycin using CLSM and FACS techniques in different cell lines. Better cellular uptake of anti-HIV drug zidovudine and lamivudine in MT-2 cell line from ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

4. Delivery of antibiotics

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. CLSM 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.

5. Transdermal Delivery of Hormones

The oral route is the commonest and convenient route of drug administration, however, administration of hormones presents with problems like high first-pass metabolism, low oral bioavailability and several dose dependent side effects like virilization, acne, and gynecomastia. these side effects reduce patient compliance increasing the risk of treatment failure. Insulin usual delivery is invasive due to its git associated problems. Thus non-invasive delivery of proteins is a better option for overcoming the problems associated with oral delivery. In 1999, Dkeidek and Touitou86 investigated the in-vivo effect of ethosomal insulin formulation in lowering blood glucose levels using normal and diabetic SDI rats. A Hill Top patch containing insulin ethosomes was applied on the abdominal area of an overnight fated rat. it was observed that that insulin delivered from this patch produced a significant decrease (up to 60%) in BGL in both normal and diabetic rats. While insulin application from a control formulation was not able to reduce the BGL.

In 2000 Touitouet al87conducted a comparative permeability study of testosterone ethosomes with a marketed transdermal patch of testosterone (Testoderm® patch, Alza Corporation, California) across rabbit pinna skin. It was observed that the ethosomal formulation had about 30-times higher skin permeation compared to that marketed formulation. With significantly improved pharmacokinetic parameters like AUC and Cmax of ethosomalTestosome as compared to Testoderm.

6. Delivery of Anti-Viral Drugs

In 2007 Mishra et al., reported ethosomes for transcutaneous immunization. Antigen-loaded ethosomes for transcutaneous immunization against Hepatitis B were prepared and characterized; they showed greater entrapment efficiency, optimal size range, and a unilamellar, spherical shape compared to conventional liposomes. Spectral bio-imaging and flow cytometric studies showed an efficient uptake by murine dendritic cells in-vitro, reaching a peak by 180 minutes. Using human cadaver skin, the transcutaneous delivery potential of the antigen-loaded antigen ethosomes demonstrated a much higher skin permeation of the antigen in comparison to the conventional liposomes and soluble antigen preparation. The topically applied HBsAg-loaded ethosomes in mice showed a robust systemic and mucosal humoral immune response compared to the intramuscularly administered alum-adsorbed HBsAg suspension, the topically applied plain HBsAg solution, and the hydroethanolic (25%) HBsAg solution. HBsAg loaded ethosomes are able to generate a protective immune response and their ability to transverse and target the immunological milieu of the skin finds a potential application in the development of a transcutaneous vaccine against Hepatitis B virus. 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 effect94,95 The optimized ethosomal formulation exhibited a transdermal flux of 78.5±2.5 mg/cm2/h across rat skin, while the hydroethanolic solution gave a flux of only 5.2±0.5 mg/cm2/h of zidovudine. The flux from ethanolic solution was found to be 7.2±0.6 mg/cm2/h. Jain et al 52 concluded from this study that ethosomes could increase the transdermal flux, prolong the release and present an attractive route for sustained delivery of zidovudine. Acyclovir is another anti-viral drug that widely used topically for treatment of Herpes labialis. The conventional marketed acyclovir external formulation is associated with poor skin penetration of hydrophilic acyclovir to dermal layer resulting in weak therapeutic efficacy 98. It is reported that the replication of virus takes place in the basal dermis. To overcome the problem associated with the conventional topical preparation of acyclovir, Horwitz et al. 47 formulated the acyclovir ethosomal formulation for dermal delivery. They have clinically evaluated its performance in a double-blind, randomized study with a marketed formulation of acyclovir (Zovirax, Glaxo Wellcome) in terms of time to crust formation, time to loss of crust and proportions of lesions not progressbeyond the popular stage (abortive lesions). Significant improvement in all evaluated clinical parameters was observed when the disorder was treated with the ethosomal formulation in comparison to the marketed formulation. The average time to crusting of lesions was 1.6 vs 4.3 days in the parallel arm and 1.8 vs. 3.5 days in the crossover arm ($P<0.025$) for ethosomal acyclovir and Zovirax, respectively. Hence, shorter healing time and the higher percentage of abortive lesions were observed when acyclovir was loaded into ethosomes.

7. Delivery of antipsoriatic and antineoplastic agent

Dubey et al. 2007 evaluated methotrexate an antipsoriatic, anti-neoplastic, highly hydrosoluble agent with limited transdermal permeation.They developed optimized ethosomes-loaded methotrexate and the skin permeation of the developed formulation revealed an enhanced permeation of rhodamine red loaded formulation to the deeper layers of the skin. The formulation retained its penetration power after storage and the vesicle skin interaction study also highlighted the penetration enhancing an effect of ethosomes, with some visual penetration pathways and corneocyte swelling[64] Delivery of Atopic Dermatitis.In 2012 Li et al prepared and evaluated tacrolimus loaded ethosomes: physicochemical characterization and in-vivo evaluation.Tacrolimus, an immunosuppressant for treating atopic dermatitis (AD), they investigated inhibition action upon allergic reactions of mice aiming at improving pharmacological effect for tacrolimus in that commercial tacrolimus ointment with poor penetration capability exhibited weak impact on AD compared with common glucocorticoid. Results showed that the ethosomes showed lower vesicle size and higher encapsulation efficiency (EE) as compared with traditional liposomes with cholesterol. Additionally, the quantity of tacrolimus remaining in the epidermis at the end of the 24-h experiment was statistically significantly greater from the ethosomal delivery system than from commercial ointment ($p<0.01$), suggesting the greater penetration ability to the deep strata of the skin for ethosomes. Interestingly, tacrolimus-loaded ethosomes with ethanol, in contrast to that with propylene glycol, showed relatively higher penetration activity except for in significant differences in EE and polydispersity index.

8. Topical application

Tacrolimus displayed the lowest ear swelling in BALB/c mice model induced by repeated topical application of 2,4-dinitrofluorobenzene compared to traditional liposomes and commercial ointment and effectively impeded accumulation of mast cells in the ear of the mice, suggesting efficient suppression for the allergic reactions. They concluded that the ethosomaltacrolimus delivery systems may be a promising candidate for topical delivery of tacrolimus in the treatment of AD102

9. Pilosebaceous targeting

In 2004, Maiden et al., prepared and evaluated the minoxidilethosomal formulation. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness. The conventional topical formulation has very poor skin permeation and retention properties. It was reported that the quantity of minoxidil accumulated into nude mice skin after application of its ethosomal formulation was 2.0-, 7.0-, and 5.0-fold higher when compared to ethanolic phospholipid dispersion, hydroethanolic solution, an ethanolic solution of the drug, each containing 0.5% of the drug. These results indicated the possibility of using ethosomes for pilosebaceous targeting of minoxidil to achieve better clinical efficacy103. it is immunologically active and thus able to express the genes. In relation to the afore mentioned facts a potential application of ethosomes, is in the topical delivery of DNA.

10. Transdermal delivery of challenging drugs

Additional studies on improved transdermal delivery by ethosomes have been cited in the literature. 1n 2005 Paolino et al., 108 evaluated the potential application of ethosomes for dermal delivery of ammonium glycyrrhizinate. Ammonium is useful for the treatment of various inflammatory based skin diseases. In-vitro skin permeation experiments have shown that a significantly higher cumulative amount of drug has permeated from ethosomes (63.2%) than from the hydroalcoholic solution (22.3%) and the aqueous solution (8.9%) of ammonium glycyrrhizinate.Ethosomal formulation showed a very good skin tolerability in human volunteers for 48-hour application. Biological anti-edema activity was also significantly enhanced in case of an ethosomal formulation as compared to an ethanolic or aqueous solution of the drug. Marketed products of ethosomal technology from 2000, when Touitou et al., discovered ethosomes commercialization of the technology began. Only two companies which developed ethosome products (Verma and Pathak, 2010).

11. Delivery of problematic drug molecules

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

12. In Cosmaceuticals

The advantage of applying ethosomes in cosmaceuticals is not only to increase the stability of the cosmetic chemicals and decrease skin irritation from the irritating cosmetic chemicals, but also for transdermal permeation enhancement, especially in the elastic forms. However, the compositions and sizes of the vesicles are the main factors to be considered to obtain these advantages of the elastic vesicles for cosmaceuticals applications.

EVALUATION PARAMETERS OF ETHOSOMES[34-42]

1. Shape and size of vesicle

The shape of the vesicle can be done by visualization using Scanning Electron Microscopy & Transmission Electron Microscopy and size of vesicles is characterized by Dynamic light scattering method and photon correlation spectroscopy. Ethosomes generally exhibit vesicular structure ranging at a size of nanometer to micrometer.

2. Zeta potential

This is usually performed using zeta meter to determine the particle surface which is used to predict the stability of ethosomes.

3. Drug entrapment

The chemical nature of the lipid is a vital factor influencing the entrapment of drug. Drug entrapment can be measured by ultracentrifugation technique, Mini column centrifugation method and also by fluorescence spectrophotometer.

4. **Vesicle skin interaction study**

This is performed by confocal laser scanning microscopy, fluorescence microscopy, Transmission Electron Microscopy, Eosin Hematoxylin Staining methods to determine the interaction of the vesicle with skin, permeation and Penetration of drug through skin.

5. **Phospholipid – ethanol interaction**

The interaction or the compatibility of Phospholipids with ethanol is determined by 319nmr and by Differential Scanning Colorimetric method.

6. **Deformability**

The degree of Deformability can be measured by extrusion method. Drug release In vitro drug release of ethosome is done by using Franz diffusion cell with artificial or biological membrane or by dialysis bag diffusion method.

7. **Drug content**

The content of the drug in the ethosome is determined by HPLC method. Surface tension activity measurement Du nuoy ring tensiometer is used to determine the surface tension of drug in aqueous solution.

8. **Stability of Ethosome**

The stability of ethosome24 is evaluated based on the entrapment efficiency and the vesicle size. This depends on the lipid composition of the ethosomes. On storage, the ethosomes undergo many changes like fusing together to form a bigger vesicle or breakdown and release the drug from the vesicle. Increase in concentration of ethanol (>45%) may lead to formation of leaky vesicle which lead to decrease in entrapment efficiency25 resulting in destabilization of ethosomes.

9. Filter Membrane-Vesicle Interaction Study

The study is done by Scanning Electron Microscopy. It involves application of vesicle suspension (0.2 mL) to filter membrane having a pore size of 50 nm and placing it in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with phosphate buffer saline solution, (having pH 6.5).The filters were removed after 1 hour and were prepared for SEM studies by fixation at 4°C in Karnovsky's fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% v/v in water). Finally, filters were coated with gold and examined in SEM .

10. Skin Permeation Studies

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

11. Stability Study

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

12. Vesicle-Skin Interaction Study by TEM and SEM

The method take place from animals ultra-thin sections were cut collected on formvar coated grids and examined under transmission electron microscope. The sections of skin after dehydration were mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater. The sections were examined under scanning electron microscope.

13. Vesicle-Skin Interaction Study by Fluorescence

Microscopy Fluorescence microscopy was carried according to the protocol used for TEM and SEM study. Paraffin blocks are used, were made, 5-µm thick sections were cut using microtome and examined under a fluorescence micro Cytotoxicity Assay MT-2 cells (T-lymphoid cell lines) were propagated in Dulbecco's modified Eagle medium containing 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mol/L Lglutamine at 37°C under a 5% CO2 atmosphere.

14. Drug Uptake Studies

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

15. HPLC Assay

The amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cell was determined by HPLC assay using methanol: distilled-water:acetonitrile (70:20:10 vol/vol) mixture as mobile phase delivered at 1mL/min by LC 10-AT vp pump. A twenty-microliter injection was eluted in C-18 column (4.6×150mm, Luna, 54, Shimadzu) at room temperature. The column eluent was monitor using SPDM10A diode array UV detector.

16. Statistical Analysis

Statistical significance of all the data generated was tested by employing ANOVA followed by studentized range test. A confidence limit < 0.05 was fixed for interpretation of the results using the software PRISM (GraphPad, Version 2.01, and San Diego, CA).

SAFETY STUDIES IN ETHOSOMES

1. In vitro studies on cultured cells

Ethosomal carriers were not toxic to 3T3 fibroblasts and the cultured cells kept their viability as assessed in a live/dead viability/cytotoxicity viability test.

2. Studies in animals

No acute skin irritation in rabbits was observed following a single-dose, 48-h, occlusive application of patches containing the ethosomal systems.

No erythema was generated following cumulative 14 day repeated ethosomal patch in rabbits. Studies in humans.

No significant variations in erythema index were measured between skin areas treated with ethosomes compared with saline.

3. Data from clinical trials

No adverse skin reactions were associated with the treatment in clinical trials with ethosomal preparations.

4. Postmarketing information

No reported adverse reaction with marketed ethosomal formulations.

FUTURE PROSPECTS OF ETHOSOMES

1. Therapeutic efficacy of topically applied drugs is often not achieved, due to the resistance of the stratum corneum to their transport into the deep skin strata.
2. Ethosomes are proposed as safe and efficient carriers for a wide range of therapeutic agents aiming at new and advanced dermal therapies.
3. Future research directions with ethosomes include delivery of biotechnological active agents, noninvasive treatment of skin cancers, noninvasive antirheumatic therapies and skin immunization.
4. The goal of future therapy should not only be restricted to costly new medications, but also to create strategies for improving the curative potential of currently used drugs.

MARKETED ETHOSOMAL PRODUCTS[33,37]

S.No	Name Of Product	Uses	Manufacturer
1.	Nanominox	First minoxidil containing ethosomes product. Contains 4% minoxidil, well-known hair growth promoter metabolized by sulfation to the active compound.	Sincere, Germany.
2.	Decorin cream	Anti-aging cream treating and repairing delayed the visible aging signs of the skin includes wrinkle lines.	Genome cosmetics, Pennyslvania, us.
3.	Supravir cream	Treatment of herpes virus.	Trima, Israel.
4.	Cellutight EF	Topical cellulite cream contains combination of ingredients to increase metabolism and breakdown of fat.	Hampden Health, USA
5.	Skin genuity	Powerful cellulite buster which reduces orange peel.	Physonics, Nottingham, UK.
6.	noicellex	Topican anti-celllite cream.	Novel therapeutics technologies, Israel.
7.	Body shape (Maccabi-CARE)	Gel executive solidification Cellulite reduction, stretching the skin flexible	Maccabi-CARE.
8.	Osmotic Lipoducton cellulite cream	To reduce cellulite and burn fat when applied to the skin.	Osmotics, Israel.

Table no.4: Marketed products of ethosomes.

CONCLUSION

Ethosomes have been found to be much more efficient at delivering drug to the skin, it can be easily concluded that ethosomes can provide better skin permeation than liposomes. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. Ethosomes are interesting and innovative vesicular systems that have appeared in the field of pharmaceutical technology and drug delivery in recent years. This carrier presents interesting features correlated with its ability to permeate intact through the human skin due to its high deformity. Therapeutic Applications of Ethosomes can be used for various purposes in drug delivery. Mainly they used as a replacement of liposomes. Ethosomes can be used for the transdermal delivery of hydrophilic and impermeable drugs through the skin.

ACKNOWLEDGEMENT

I would like to express my a deep sense of gratitude of thankfulness to Mr. Dilip Kumar Tiwari for his valuable guidance felicitous advice during the course of this work.

REFERENCES

[1] Gangwar S., Singh S., Garg G., Ethosomes: A Novel tool for Drug Delivery through the Skin, Journal of Pharmacy Research 2010;3,4:688-691.

[2] Kumar KP., Radhika P.R., Sivakumar T., Ethosomes: a priority intransdermal drug delivery system, International journal of advances in pharmaceutical sciences 2010;1:111-121.

[3] Jain H., Patel J., Joshi K., Patel P., Upadhyay UM., Ethosomes: A Novel Drug Carrier, International Journal of Clinical Practice 2011;7:1:1-4.

[4] Upadhyay N., Mandal S., Bhatia L., Shailesh S., Chauhan P., A Review on Ethosomes: An Emerging Approach for Drug Delivery through the Skin, Recent Research in Science and Technology 2011;3,7:19-24. Divya * et al./ Int. J. Pharm. Med. Res. 2016; 4(4):354-363 362.

[5] Sivakranth M., AnjumaAra P., Krishnaveni C., Venkatesh E., Ethosomes: A Novel Vesicular Drug Delivery System, International Journal of Advances in Pharmaceutical Research 2012;2,1:16-27.

[6] Kumar R., Aslam MD., Tripathi A., Prasad D., Chaudhary V., Jain V., Mishra SK., Singh R., Ethosomes: Novel Vesicular Carriers in Transdermal Drug Delivery, Journal of Global Pharma Technology 2010;2,6:1-7.

[7] Rathore AR., Khambete H., Jain S., Preparation and Characterization of Repaglinide Loaded Ethosomal Gel for the Treatment of NIDDM, International Journal of Pharmaceutical and Biological Archives 2013;4,2:385- 390.

[8] Shahwal V., Samnani A., Dubey B., Bhowmick M., Ethosomes: An Overview, International Journal of Biomedical and Advance Research 2011;2: 161-168.

[9] Touitou E., Drug delivery Across Skin, Expert Opinion on Biological Therapy 2002;2:723-733.

[10] Schreier H., Bovwstra J., Liposomes and Niosomes as Topical Drug Carriers: Dermal and Transdermal Drug Delivery, Journal of Control Release 1994;30:1-15.

[11] Dinesh D., Amit AR., Maria S., Awaroop RL., Mohd Hassan GD., Drug Vehicle Based Approaches of Penetration Enhancement, International Journal of Pharmacy and Pharmaceutical Sciences 2009;11:24- 45.

[12] Verma P., Pathak K., Therapeutic and cosmeceutical potential of ethosomes: An overview, Journal of Advanced Pharmaceutical Technology & Research 2010;1:3:274–282.

[13] Jain S., Umamaheshwari RB., Bhadra D., Jain NK., Ethosomes: A Novel Vesicular Carrier for Enhanced Transdermal Delivery of a Anti-HIV Agent, Indian Journal of Pharmaceutical Sciences 2004;66:72-81.

[14] Verma DD., Fahr A., Synergistic Penetration Effect of Ethanol and Phospholipids on the Topical Delivery of Cyclosporin, Journal of Controlled Release;97:55-66.

[15] Touitou E., Composition of Applying Active Substance to or Through the Skin, US patent:5,540,934,1998.

[16] Rao Y., Zheng F., Zhang X., In-Vitro Percutaneous Permeation and Skin Accumulation of Finasteride Using Vesicular Ethosomal Carriers, AAPS Pharm Sci Tech 2008;9:860-865.

[17] Pratima NA., Tiwari S., Ethosomes: A Novel Tool for Transdermal Drug Delivery, International Journal of Research in Pharmacy and Sciences, 2012;2,1:1-20.

[18] Celia C., Cilurzo F., Trapasso E., Cosco D., Fresta M., Paolino D., Ethosomes and Transfersomes Containing Linoleic Acid: Physicochemical And Technological Features of Topical Drug Delivery Carriers For The Potential Treatment of Melasma Disorders, Biomedical Microdevices 2011;6:105-111.
[19] Biju SS., Sushama T., Mishra PR., Khar PR., Vesicular Systems: An Overview, Indian Journal of Pharmaceutical Sciences 2006;682: 141-153.
[20] Touitou E., Godin B., Dayan N., Weiss C., Piliponsky A., Levi-Schaffer F., Intracellular Delivery Mediated by an Ethosomal Carrier, Biomaterials 2001;22:3053-3059.
[21] Jain S., Jain P., Jain NK., Transfersomes: A Novel Vesicular Carrier for Enhanced Transdermal Delivery, Development, Characterization and Performance Evaluation, Drug Development and Industrial Pharmacy 2003;29:1013–1026.
[22] Sivakranth M., Ara PA., Krishnaveni C., Venkatesh E. Ethosomes a Novel Vesicular Drug Delivery System, International Journal of Advances in Pharmaceutical Sciences 2012;21:16-27.
[23] Manosrai A., Jantrawut P., Khositsuntiwon N., Manosroi W., Manosroi J., Novel Elastic Nanovesicles for Cosmeceutical and Pharmaceutical Applications, J Sci. 2009;36:168-78
[24] Kulkarni RV., Doddayya H., In-vitro Permeation of Verapamil Hydrochloride From Polymeric Membrane Systems Across Rat and Human Cadaver Skin, Indian Journal of Pharmaceutical Sciences 2002;593-597.
[25] Kaur R., Agrawal SS., Development and Evaluation of Transdermal Delivery System of Cavediol, Sci Abs. 54 th IPC 2002;21.
[26] Patel MM., Sheth MN., Harinawala AI., Studies in The Transdermal Formulations of Metoprolol Tartrate and Their Evaluation Using Human Cadaver Skin, The East Pharmacist1993;129-131.
[27] Gangwar S., Ethosomes: For Drug Delivery, Journal of Pharmacy Research 2010;3,4:688-691.
[28] Kumar KP., Radhika PR., Sivakumar T., Ethosomes: A Priority in Transdermal Drug Delivery, International Journal of Advances in Pharmaceutical Sciences 2010;1:111-121.
[29] Tyagi LK., Kumar S., Ethosomes: Novel Vesicular Carrier For Enhanced Transdermal Drug Delivery System, Bulletin of Pharmaceutical Research 2013;31:6- 13.
[30] Grace X Fatima*, Raj S. Rahul, I. Reshma, T.Sandeep, S.Shanmuganathan and D.Chamundeeswari. Review Article Herbal Ethosomes- A Novel Approach in Herbal Drug Technology. American Journal of Ethnomedicine, 2014, Vol.1, No.4, 226-230.
[31] Pawar Priyanka*, Kalamkar Ranjan, Jain Ashish, Amberkar Shoyab. Review Article Ethosomes: A Novel Tool For Herbal Drug Delivery. International Journal of Pharmacy & Pharmaceutical Research, July 2015 Vol.:3, Issue: 4: 191-202.
[32] Aggarwal Divya*, Nautiyal Ujjwal. Ethosomes: A Review. International Journal of Pharmaceutical and Medicinal Research, 2016; 4(4):354-363, Issn: 2347-7008.
[33] Patel D.* and Bhargava P. Review Article, Ethosomes- A Phyto Drug Delivery System. Advanced Research in Pharmaceuticals and Biologicals, 2012; Vol: 2 (1) jan-march. Issn: 2250-0774.
[34] Patrekar Prasad V. *, Inamdar Suhel J, Mali Sachin S, et al. Ethosomes as novel drug delivery system: A Review, The Pharma Innovation Journal 2015;4(9): 10-21, Issn: 2277-7695.
[35] Petty M. Miyanda *, S. Priyanka, Gautam Surya. Review Article Ethosomes: a potential transdermal drug delivery system in depth review, International Journal of Pharmacy & Pharmaceutical Research, December 2017 Vol.:11, Issue:1 14-45, Issn: 2349-7203.
[36] Agrawal S.*, Gautam G, Rajpoot N. A Review on rthosomes: an advanced approach to transdermal drug delivery system, International Research Journal of Pharmacy 2019, 10(4) Issn: 2230-8407.
[37] Kumar A. Eswar*. AReview on novel trends in vesicular carrier for enhanced transdermal delivery: ethosomes,International Journal of Scientific Research, Vol. 6, (4) April 2017, Issn: 2277-8179.
[38] Tiwari Anupama*, Mishra Manoj Kumar, Nayakk Kania, et.al. Ethosomes: a novel vesicular carrier system for therapeutic applications, IOSR Journal of Pharmacy, Vol. 6, Issue 9, September 2016, 25-33.
[39] Patel Ravi B*, Patel Tejas B, Suhagia B.N, et.al. Review Article Ethosomes: an emerging targeted drug delivery system a review, International journal of innovative pharmaceutical sciences and research, 2(4),2014 941-961, Issn: 2347-2154.
[40] Pathan Inayat B, Nandure Hiraman, Syed Shoaeb Mohammad, Bairagi Shripad. Research Article: Transdermal delivery of ethosomes as a novel vesicular carrier for paroxetine hydrochloride: In vitro evaluation and Invivo study, Marmara Pharmaceutical Journal 20:1-6, 2016.
[41] Barupal A.K., Gupta Vandana, Ramteke Suman*. Research Article preparation and characterization of ethosomes for topical delivery of aceclofenac, Indian Journal of Pharmaceutical Sciences, September-october 2010.
[42] Bhagya Rekha*. Forulation and characterization of atorvastatin rthosomal gel, Innovation info, Journal of Drug Development and Delivery, 2018 Vol.1:1 Issue:1, 13-20.