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ETHOSOMES AS DRUG CARRIER: A NOVEL APPROACH

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ABSTRACT

The transdermal route vied with oral treatment as the most successful innovative research area in drug delivery. The use of lipid vesicles in delivery systems for skin treatment has attracted increasing attention in recent year. Vesicles allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response and would be able to release just the right amount of drug and keeping the concentration constant for longer period of time. Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layer and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. Ethosomes are the ethanolic phospholipid vesicles which are used mainly for transdermal delivery of drugs. Ethosomes have higher penetration rate through the skin as compared to liposomes, hence these can be used widely in place of liposomes. The increased permeation of ethosomes is probably due to its ethanolic content. Ethanol increases the cell membrane lipid fluidity which results in increased skin penetrability of the ethosomes. Evaluation parameter include size, shape, drug content, zeta potential, stability studies, skin permeation studies etc. Ethosome provides a number of important benefits including improving the drug's efficiency, enhancing patient compliance and comfort and reducing the total cost of treatment.

KEY WORDS: Transdermal delivery, Liposomes, Ethosome.

INTRODUCTION

The skin covers a total surface area of approximately 1.8$m^2$and provides the contact between the human body and the external environment. The Stratum corneum is the outermost layer of the epidermis. It consists of 10 to 25 layers of dead, elongated, fully keratinized corneocytes, which are embedded in a matrix of lipid bilayers [1-2].The stratum corneum is the main barrier to penetration through the skin. When a topical formulation is placed on the skin active drug is required to penetrate through the stratum corneum into the viable tissue. The limiting factor for these processes is the slow diffusion through the dead horny layer of skin.7-10 Stratum corneum behaves as a hydrophobic membrane. The rates of permeation of skin by low and high molecular weight organic non-electrolytes are mostly determined within the stratum corneum [3-4]. Skin forms a protecting covering layer against the external environment and prevents water loss from the underlying tissue. It is flexible enough to resist permanent distortion from movement and thin enough to allow the perception of stimuli. It also performs many ancillary functions such as synthesis and metabolism and the production of sweat enables temperature control and excretion of waste products by means sweating etc [5-6].

The skin can be considered to be composed of three layers: subcutaneous tissue, dermis and epidermis layer [7] as shown in figure 1.

The penetration through skin is also affected by several biological factors such as skin age, body site, skin condition and diseases, water content of the skin or hydration [8].

Dermal drug delivery is the topical application of drugs to the skin in the treatment of skin diseases and other inflammatory conditions. This has the advantage that high concentrations of drugs can be localized at the site of action, reducing the systemic side effects [10]

Now-a-days liposomes, niosomes, transferosomes and ethosomes (vesicular and non- invasive drug delivery) are used to increase the permeation of drug through the stratum corneum. One of the major advances in vesicle research was the finding that some modified vesicles

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possessed properties that allowed them to successfully deliver drugs in deeper layers of skin [11].

Ethosomes are novel carrier system used for delivery of drugs having low penetration through the biological membrane mainly skin.

Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water [12].

Advantages claimed are increased patient acceptability (non-invasiveness), avoidance of gastrointestinal disturbances and first pass metabolism of the drug [13].

To overcome the stratum corneum barrier, various mechanisms have been investigated, including use of chemical or physical enhancers, such as iontophoresis, sonophoresis, etc. Liposomes, niosomes, transferosomes and ethosomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier [14-15].

Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles as well as by the presence of ethanol in ethosomes which allows for better solubility of many drugs. Ethosomes were reported to improve *invivo* and *in vitro* skin delivery of various drugs. Contrary to deformable liposomes, ethosomes are able to improve skin delivery of drugs both under occlusive and non-occlusive conditions [16-17].

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

Drug and vehicle interactions

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

Stratum corneum modification

- Hydration
- Chemical penetration enhancers

Stratum corneum bypassed or removed

- Microneedle array
- Stratum corneum ablated
- Follicular delivery

Electrically assisted methods

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

Vesicles and particles

- Liposomes and other vesicles
- Niosomes
- Transfersomes
- ethosomes [18]

The size range of ethosomes may vary from tens of nanometers (nm) to microns (m) ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux [19].

Ethosomes provides a number of important benefits including improving the drug's efficacy, enhancing patient compliance and comfort and reducing the total cost of treatment. The Ethosomes were found to be suitable for various applications within the pharmaceutical, biotechnology, veterinary, cosmetic, and nutraceutical markets. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an Ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies [20].

CROSS SECTION OF HUMAN SKIN

Skin is a multilayered organ complex in both structure and function. Macroscopically, the outer epidermis and the inner dermis are two distinct layers of the skin.

The layers of epidermis are:

- Stratum Corneum (Horny Layer)
- Stratum Lucidum (Clear Layer)
- Stratum Granulosum (Granular Layer)
- Stratum Spinosum (Prickly cell Layer)
- Malpighian Layer (pigment Layer)
- Stratum Germinativum (regenerative Layer)

Epidermis is the outermost layer of the skin, which is approximately 150 micrometers thick. Cells from lowers layers of the skin travel upward during their life cycle and become flat dead cells of the corneum. The epidermis is a multilayered structure consisting of viable cells and dead keratinized cells. The layer that interacts with the environment is the stratum corneum, or horny layer. The stratum corneum consists of many layers of compact, flat, dehydrated and keratinized cells. These cells are physiologically inactive and are continuously shed with constant replacement from the underlying viable epidermal tissue. The stratum corneum has a water content of only 20% as compared to the normal physiological level of 70%, such as in the physiologically active stratum germinativum (which is the regenerative layer of the epidermis).

Stratum Corneum

The stratum corneum (10-15μm thick) is the skin’s primary defense layer against invasion .The major lipid classes within the stratum corneum are ceramides, cholesterol, and fatty acids. Their major structural components are aggregates of keratin filaments. All these contribute to tightness and impermeability characteristics of the skin.

Stratum Lucidum

In the palm of the hand and sole of the foot, a zone forms a thin, translucent layer immediately above the granule layer. The cells are non-nuclear.

Stratum Granulosum

This layer is above the keratinocytes. They manufacture the basic staining particle, the keratinohylline granules. This keratogenous or transitional zone is a region of intense biochemical activity and morphological change.

Stratum Spinosum

The cells of this layer are produced by morphological and histochemical alteration of the cells basal layers as they moved upward. The cells flatten and their nuclei shrink. They are interconnected by fine prickles and forms intercellular bridges- the desmosomes. These links maintain the integrity of the epidermis.

Malpighian Layer

The basal cells also include melanocytes which produce and distribute the melanin granules to the keratinocytes required for pigmentation - a protective measure against radiation.

Stratum Germinativum

Basal cells are nucleated, columnar. Cells of this layer have high mitotic index and constantly renew the epidermis and this proliferation in healthy skin balances the loss of dead horny cells from the skin surface.

The human skin contains the dermis, approximately 2-3 mm thick, forms the bulk of the skin. The dermis contains a network of blood vessels, lymph vessels, hair follicles, sweat glands & sebaceous glands – skin appendages.

Beneath the dermis is the hypodermis, which is primarily composed of fibroblasts and adipocytes - sub cutaneous fatty tissues. Bulbs of hair project into these fatty tissues.

The hypodermis binds skin to the underlying structures, in addition to serving as a thermo regulator and a cushion to internal organs against trauma.

The skin is interspersed with hair follicles and associated sebaceous glands and sweat glands. Collectively these are referred to as skin appendages.

On an average of 10-70 hair follicles and 200-500 sweat ducts per square centimeter are present on the skin surface. These skin appendages occupy only [21].

ETHOSOMES AS A NOVEL CARRIER

Ethosomes are noninvasive delivery carriers that enable drugs to reach the deep skin layers and/or the systemic circulation. These are soft, malleable vesicles tailored for enhanced delivery of active agents. They are composed mainly of phospholipids, (phosphatidyl choline, phosph-atidylserine, phosphatitidic acid), high concentration of ethanol and water as shown in figure 2. 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.

Ethosomes are soft, malleable vesicles composed mainly of phospholipids, ethanol (relatively high concentration) and water. These “soft vesicles” represents novel vesicular carrier for enhanced delivery to/through skin. The size of Ethosomes vesicles can be modulated from tens of nanometers to microns [22].

CHARACERISTIC FEATURES

- They are developed by TOUITOU et al in 1997.
- Size range: tens of nanometers to microns.
- Compared to conventional liposomes they permeate more rapidly and possessed higher transdermal flux.
- The synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid layers.
- Permeation enhancers are used to improve the permeability of the skin, so that the drugs can cross through the skin easily.
- Ethosomes can entrap drug molecules with various physicochemical characteristics i.e., of hydrophilic, lipophilic or amphiphilic.
- The high concentration of ethanol in ethosomes causes disturbance of skin lipid bilayer organization hence it enhances the vesicles ability to penetrate the stratum corneum.
- Also, because of their high ethanol concentration the lipid membrane is packed less tightly than the conventional vesicles, although it has equivalent stability, allowing a more malleable structure and includes the drug distribution ability in the stratum corneum lipids.
- Ethosomal system is much more efficient at delivery a fluorescent probe to the skin in terms of quantity and capacity for molecule of various lyophilicities [23].

Ethosomes Composition

The ethosomal system consists of phospholipids, ethanol and water [24].

The phospholipids with various chemical structure includes phosphatidyl choline (PC), hydrogenated PC, phosphatidyl ethanolamine(PE), phosphatidyl glycerol (PPG), phosphatidy linositol (PI), hydrogenated PC etc [25].

The non-aqueous phase range between 22 % to 70 %. The alcohol may be ethanol or isopropyl alcohol. Dyes oramphiphilic fluorescent probe such as D – 289, Rhodamine – 123, fluorescence isothiocynate (FITC), 6 –

carboxy fluorescence are often added to ethosomes for characterization study. [26-27].

ETHANOL- AS PENETRATION ENHANCER

Substances that reversibly reduce the barrier resistance of the stratum corneum are known as chemical penetration enhancers. Ethanol is one of the most commonly used permeation enhancers. A number of mechanisms have been proposed for permeation enhancing action of ethanol. As a solvent, ethanol can be included in the formulation to enhance the solubility of the drug. This is particularly important for poorly soluble permeates, as they are prone to depletion in the donor vehicle (Lodzki M, 2003). Ethanol is a relatively volatile solvent and will rapidly evaporate at skin temperature. Ethanol loss from a formulation may lead to the drug becoming supersaturated, which will influence drug flux across the membrane. In addition, ethanol is thought to alter the solubility properties of the stratum corneum, facilitating improved drug partitioning [28].

Ethanol has been employed in vitro to enhance trans-dermal delivery of levonorgesterol, hydrocortisone and 5-fluorouracil across rodent skin (Williams AC, 2003), and estradiol across human skin in vivo (Friend D, 1988). Megrab and collaborators (Megrab NA, 1995) noted that the enhancement effect of ethanol was concentration dependent. The authors investigated the effect of ethanol on skin water content and concluded that formulations containing high levels of alcohol were capable of dehydrating the skin, which may explain the concentration dependant action of ethanol [29].

Advantages of Ethosomal Drug Delivery

1. Ethosomes are enhanced permeation of drug through skin for transdermal and dermal delivery.
2. Ethosomes are platform for the delivery of large and diverse group of drugs. (peptides, protein molecules)
3. High patient compliance-The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance [30].
4. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods [31].
5. It contains non-toxic raw material in formulation.
6. The Ethosomal system is passive, non-invasive and is available for immediate commercialization [32].
7. 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.
8. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
9. Enhanced permeation of drug molecules through the skin to the systemic circulation.
10. Controry to deformation liposomes, ethosomes improve skin delivery of drugs both under occlusive and non occlusive condition.
11. Better stability and solubility of many drugs as compared to conventional vesicles.
12. Relatively smaller size as compared to conventional vesicles [33].

Disadvantages of Ethosomes

1. Drugs that require high blood levels cannot be administered – limited only to potent molecules, those requiring a daily dose of 10mg or less.
2. Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it is usually designed to offer slow, sustained drug delivery.
3. Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
4. The molecular size of the drug should be reasonable that it should be absorbed percutaneously.
5. Adhesive may not adhere well to all types of skin. Uncomfortable to wear.
6. May not be economical. Poor yield
7. Skin irritation or dermatitis due to excipients and enhancers of drug delivery systems.
8. In case if shell locking is ineffective then the ethosomes may coalescence and fall apart on transfer into water.
9. Loss of product during transfer from organic to water media.
10. The main advantage of ethosomes over liposomes is the increased permeation of the drug [34].

MECHANISM OF PENETRATION

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

This is followed by the 'ethosome effect', which includes inter lipid penetration and permeation by the opening of new pathways due to the malleability and fusion of ethosomes with skin lipids, resulting in the release of the drug in deep layers of the skin as shown in **Figure 3**.

The drug absorption probably occurs in following two phases:

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

Ethosomes effect: Increased cell membrane lipid fluidity caused by the ethanol of ethosomes results increased skin

permeability. So the ethosomes permeates very easilyinside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin [36].

PREPARATION OF ETHOSOMES

Cold Method

This is the most common method utilized for the preparation of Ethosomal formulation. In this method Phospholipid, drug and other lipid materials is mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 30^0C in a water bath. The water heated to 30^0 C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle sizes of dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of Ethosomal formulation can be decreased to desire extend using sonication or extrusion method. Finally, the formulation is stored under refrigeration.

Hot method

In this method Phospholipid is dispersed in water by heating in a water bath at 40^0C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400C. Once both mixtures reach 40^0C, 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 [38].

Dissolve phospholipid in an organic solvent or a mixture of organic solvents in a round bottom flask (RBF).remove the organic solvent using a rotory vaccum evaporator above lipid transition temperature to form a thin lipid film on the wall of the RBF. Traces of the solvent should be removed from the deposited lipid film by leaving the contents under vaccum overnight. Hydrate the lipid film with hydroethanolic solution of drug by rotating the flask at suitable temperatures with or without intermittent sonication and finally, cool the resultant ethosomal suspension at room temperature the formulation should be stored under refrigeration.

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Classic method

The phospholipid and drug are dissolved in ethanol and heated to 30^0 $c^{+/-}1^o$ c in 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.

CHARACTERIZATIONS OF ETHOSOMES:

Visualization

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

Vesicle size and Zeta potential

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

Entrapment Efficiency

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

Transition Temperature

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

Surface Tension Activity Measurement

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

Vesicle Stability

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

Penetration and Permeation Studies

Depth of penetration from ethosomes canbe visualized by
confocal laser scanningmicroscopy (CLSM) [39].

Degree of deformability

The elasticity of ethosomal vesicle membrane can be determined by extrusion method. The ethosomal formulation is extruded through the filter membrane (pore diameter 50nm) using stainless steel filter holder of diameter 25nm, by applying a pressure of 2.5bar.

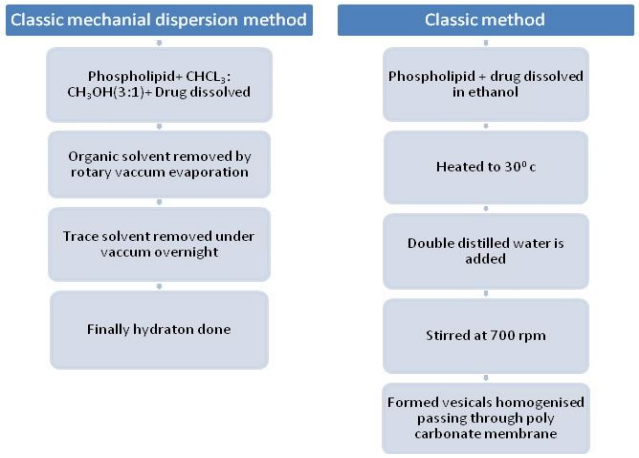
Turbidity

It can be measured by nephloturbidometer [40].

EVALUATION TESTS

Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy

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



Skin Permeation Studies

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

Comparison of in vitro skin permeation of drug forms various formulations:

Invitro skin permeation of diclofenac potassium in ethosomes, liposomes, hydroethanolic solution (1%w/v) and in phosphate buffer saline pH 7.4 (1%w/v) were studied using Franz diffusion cell with an effective permeation area of 2.54cm^2. The ethosomal formulation was selected for the in vitro skin permeation on the basis of high entrapment efficiency and smaller vesicular size. Rats (male albino) 6 to 8 weeks old, weighing 120 to 150g were sacrificed for abdominal skin. After removing the hair, the abdominal skin was separated from the underlying connective tissue with scalpel. The excised skin was placed on aluminum foil and the dermal side of the skin was gently teared off for any adhering fat and/or subcutaneous tissue. The skin was checked carefully to ensure the skin samples are free from any surface irregularity such as fine holes or crevices in the portion that is used for transdermal permeation studies. The in vitro study was approved by the institutional ethical committee. The skin was mounted between donor and receptor compartment with the stratum corneum side facing upward into the donor compartment. Phosphate buffer saline pH 7.4 was taken in the receptor compartment. The formulation was applied on the skin in donor compartment which was then covered with aluminum foil to avoid any evaporation process. Samples were withdrawn at predetermined time intervals over 12 hours, and suitably diluted with phosphate buffer saline pH 7.4 to analyze the drug content in UV-Visible spectrophotometer at 276nm using phosphate buffer saline pH 7.4 as blank. The receptor medium was immediately replenished with equal volume of fresh medium to maintain the sink conditions throughout the experiment. The percentage of drug release was plotted against time to find the drug release pattern.

FTIR studies: - Stability studies were carried out by storing the ethosomal formulations at two different temperatures 4ºC and 25±2ºC. The drug content was estimated for every 15 days to identify any change in the entrapment efficiency of ethosomal formulation **[**41].

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.

Vesicle Skin Interaction Study by TEM and SEM

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

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 (Erma optical works, Tokyo, Japan) and

examined under a fluorescence micro Cytotoxicity Assay. MT-2 cells (T-lymphoid cell lines) were propagated in Dulbecco's modified Eagle medium (HIMEDIA, Mumbai, India)containing 10% fetal calf serum, 100 U/Ml penicillin, 100 mg/mL streptomycin, and 2mmol/L L-glutamine at 37°C under a 5%CO2 atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at 540nm.

Drug Uptake Studies

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

HPLC Assay

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

Statistical Analysis

Statistical significance of all the data generated was tested by employing ANOVA followed by student zed range test. A confidence limit of $P < .05$ was fixed for interpretation of the results using the software PRISM (GraphPad, Version2.01, San Diego, CA) [42]

APPLICATION OF ETHOSOME

1) Pilosebaceous Targeting

Hair follicles and sebaceous glands are increasingly being recognized as potentially significant elements in the percutaneous drug delivery. Furthermore considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery.

2) 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. The risk of failure of treatment is known to increase with each pill missed.

3) 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 MI muscarinic receptors antagonist and used in the treatment of Parkinson disease. The results indicated better skin permeation potential of ethosomal-THP formulation and its use for better management of Parkinson disease.

4) Transcellular delivery

Touitou et al. In their 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 to be an attractive clinical alternative for anti-HIV therapy.

5) 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-weeks male CD-1 nude mice for 48hr. 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. These results also showed the possibility of using ethosomes for effective transdermal formulation. Hence, better skin permeation ability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents.

6) Delivery of anti-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. Lodzki et al. Prepared CBD-ethosomal formulation for transdermal delivery. Results shows significantly increased in biological anti- inflammatory activity of CBD-ethosomal formulation was observed when tested by carrageenan induced rat paw edema model. It was concluded encapsulation of CBD in ethosomes significantly increased its skin permeation, accumulation and hence it's biological activity.

7) Delivery of antibiotics

Topical delivery of antibiotics is a better choice for increasing the therapeutic efficiency of these agents.

Conventional oral therapy causes several allergic reaction along with the several side effects. Conventional external preparation possess low permeability to deep skin layers and sub-dermal tissues. Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotics into the deeper layer of the skin. Ethosomes penetrates 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. 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.

8) Delivery of anti-viral drugs

Zidovudine is a potent anti-viral 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. Jain *et al.*, concluded 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 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. The results showed that shorter healing time and higher percentage of abortive lesions were observed when acyclovir was loaded into ethosomes [43].

9) Ethosomes are mainly used as replacement of liposomes.

10) Ethosomes, the high ethanol containing vesicles are able to penetrate the deeper layer of the skin and hence appear to be ethosomes choose for the transdermal delivery of hydrophilic and impermeable drugs through the skin. Various drugs have been used with ethosomal carrier [44].

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) Patented and marketed formulation of ethosome

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

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 control over drug release *in vivo*, allowing physician to make the therapy more effective. Ethosomes offers a good opportunity for the non-invasive delivery of small, medium and large sized drug molecules. The results of the first clinical study of acyclovir-ethosomal formulation support this conclusion. Multi liter quantities of ethosomal formulation can be prepared very easily. It, therefore, should be not before long that the corresponding drug formulation would have found their way into clinics to be tested for widespread usage. Thus, it can be a logical conclusion that ethosomal formulations possess promising future in effective dermal/transdermal delivery of bioactive agents [45].

Table 1. Different additives employed in formulation of Ethosomes

Class	***Example***	***Uses***
Phospholipid	Soya phosphatidyl choline, Egg phosphatidyl choline Dipalmityl phosphatidyl choline, Distearyl phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Transcutol RTM	As a skin penetration enhancer
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane, As a penetration enhancer

Cholesterol	Cholesterol	For providing the stability to vesicle membrane
Dye	Rhodamine-123 Rhodamine red, Fluorescene Isothiocynate (FITC),6- Carboxy fluorescence	For characterization study
Vehicle	Carbopol Đ934	As a gel former

Table 2. Application of Ethosomes as a Drug Carrier Drugs Results

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

Fig 1. Structure of skin

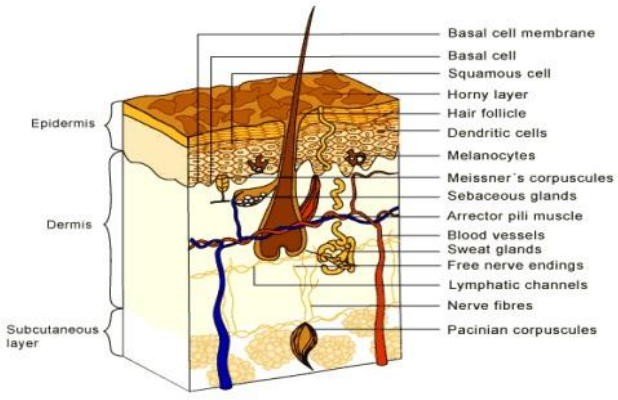


Fig 2. Structure of Ethosomes

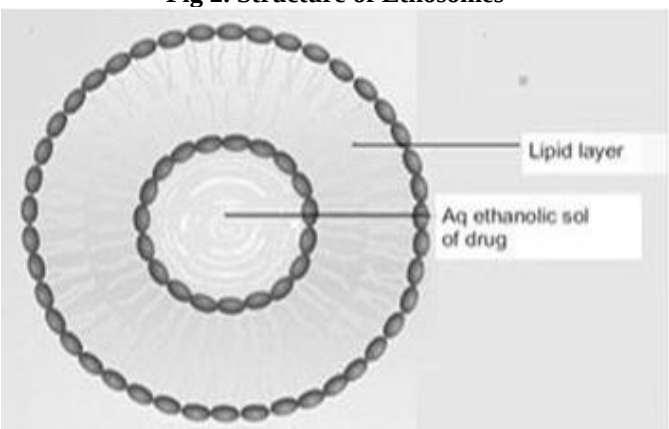
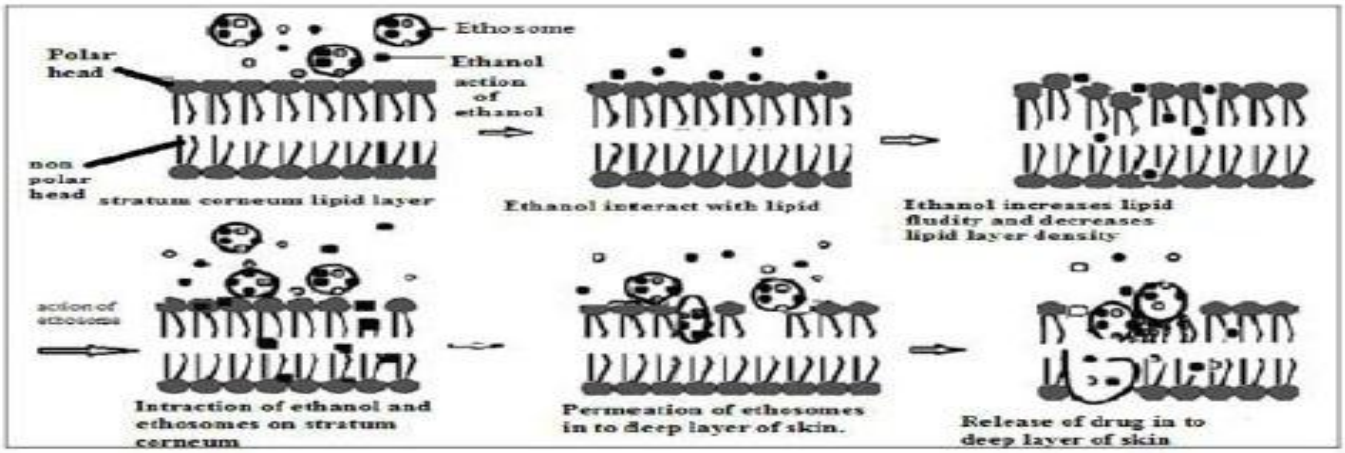
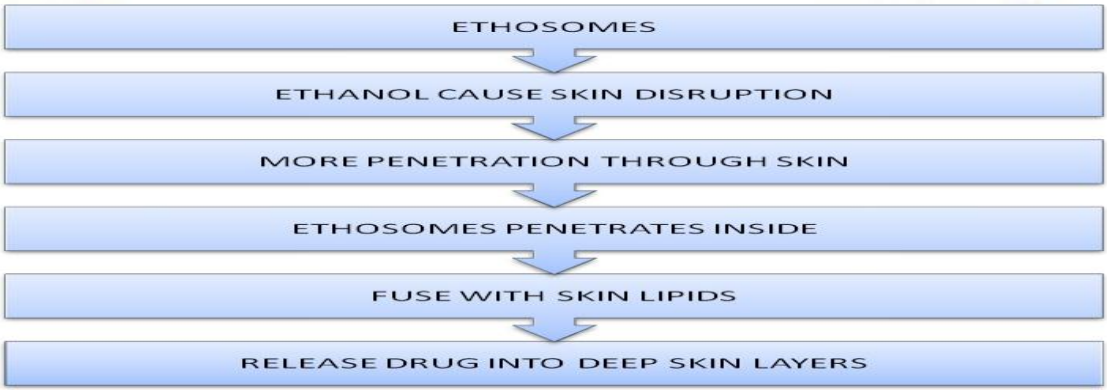
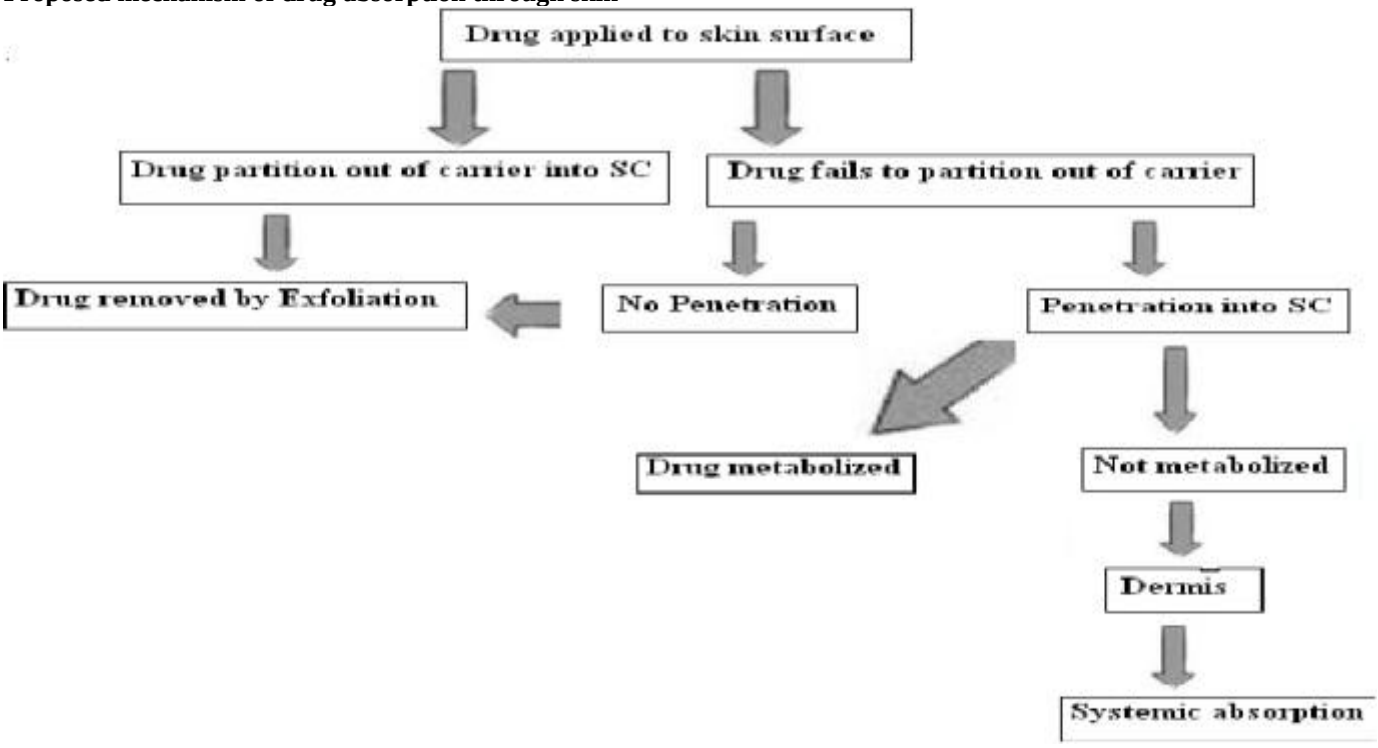


Fig 3. Mechanism of action of ethosomes

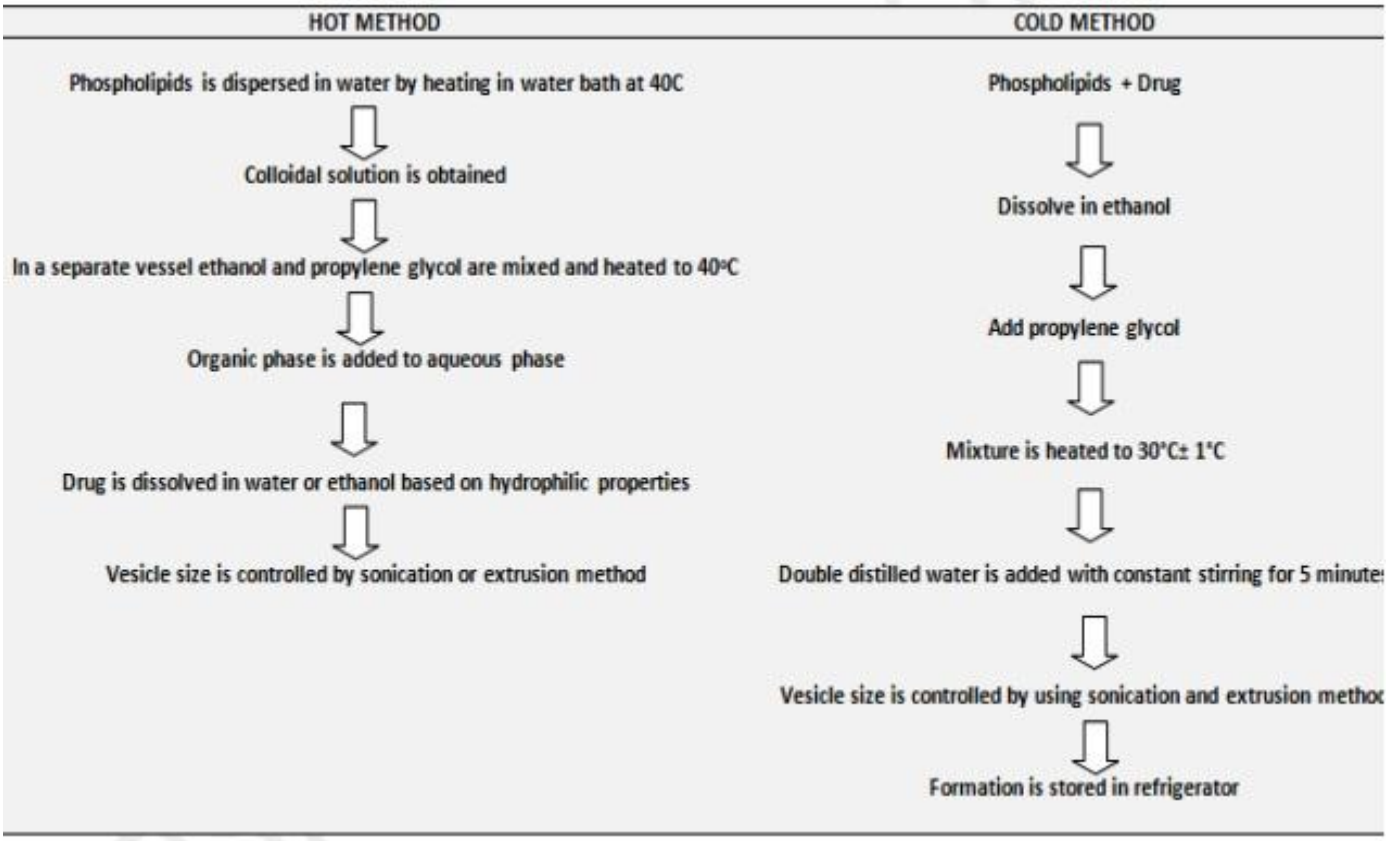




Proposed mechanism of drug absorption through skin



Classic mechanical dispersion method



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