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AN OVERVIEW ON ETHOSOMES: NEED OF FUTURE

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1. Abstract:

Delivery through skin is still difficult to many drug molecules because of its molecular size, weight, its solubility which also affects drug penetrability, diffusion and its bioavailability. Skin is also act as barrier to the molecules. Past few decades, various successful techniques and devices have emerged to optimize drug delivery across skin. Ethosome is one of these techniques which have been recently emerged as great advantage for drug moieties. Ethosomes are ethanolic phospholipid vesicles and modified form of liposomes mainly for transdermal delivery of drugs. Although ethosomes are conceptually sophisticated, they are characterized by simplicity in their formulation, safety, and efficacy. Ethosomes are soft vesicles prepared for enhanced delivery of active agents. Ethosomes have higher penetrability rate through the skin as compare to other nanoparticles. This increased permeability is because of ethanolic content as ethanol itself act as penetration enhancer gives synergistic effect. Ethanol enhances the cell membrane lipid fluidity results in increased skin penetrability of the ethosomes. These ethosomes permeates through the skin and fuse with cell membrane lipids and releases the drug. Cold and hot are the main methods used for formulation of ethosomes. Evaluation parameters include vesicle size, shape, drug content, zeta potential, stability etc. The following article reviews in detail about ethosomes mechanism, methods of preparation, evaluation parameter, application and future of ethosomes in pharmaceutical, cosmetic and nutraceutical industry. Improved delivery of drug molecules through the skin and cellular membranes by ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

Keywords: Ethosomes, penetration enhancer, phospholipid, transferosomes

2. Introduction:

Transdermal delivery of drugs offers many advantages compared to traditional delivery system like increase patient compatibility, avoid GI disturbance and avoid first pass metabolism of drug by introducing the drug directly from site of action to systemic circulation [1,2]. Administration of drug through skin route gives many advantages but the barrier nature of skin makes hard to pass most of the drugs to penetrate and permeate to skin [3]. Skin composed of mainly 3 layers such as epidermis layer, dermis and subcutaneous layer. Epidermis layer is made of 5-layer stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum and stratum basale. Stratum corneum is the outermost layer of epidermis. It decides the permeation of low and high molecular weight of drugs. The main route of penetration of drug is at the lipid bilayer [4]. When it comes to herbal drugs, the it is difficult to administer herbal drug through skin because of their solubility, particle size and their complexity. Herbal drugs are mostly administering through transdermal route. Since past years, there are many new formulation techniques of drug delivery system to overcome the stratum corneum barrier. Techniques like sonophoresis, iontophoresis electroporation lipid vesicles like liposomes, noisome, phytosomes, transferosomes and ethosomes etc. [5,6]. Drug encapsulated in lipid vesicles prepared from phospholipid gives nontoxic penetration of drugs in to the stratum corneum because of its lipophilic nature. Lipid rich vesicles such as niosomes, liposomes, ethosomes and phytosomes used for encapsulation of hydrophilic as well as hydrophobic drugs also drugs with low and high molecular weight [7,8]. But sometimes these lipids-based vesicles have problem with poor skin permeability. So, the introduction ethosomes which are another novel lipid carrier developed by Touitou *et al* has shown enhanced skin permeation as compared to conventional lipid vesicles [2,9].

3. Ethosomes:

Ethosomes are lipid vesicles containing phospholipid, alcohol and water. Alcohol is generally ethanol or isopropyl alcohol in high concentration as compare to water. This ethanol containing lipid vesicular system was invented by Touitou. Ethosomes are modified form of well-established liposomes. The basic difference between liposomes and ethosomes is ethosomes contains ethanol. Ethosomes have reported to increases the permeability of many drugs. Ethosomes have proved themselves as a good delivery carrier in transdermal delivery system and its enhancement effect has been recognized widely. The presence of ethanol in lipid bilayer improves carrier penetration through stratum corneum layer allows efficacious local and systemic delivery of both hydrophilic and lipophilic compounds [10-13].

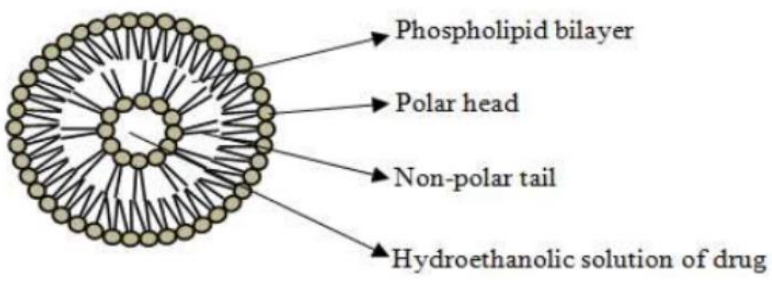

Fig. 1: Structure of an Ethosome

Size ranges from tens of nanometer (nm) to microns (μ). Ethosomes permeate rapidly through skin and having significantly higher transdermal flux. Liposomes which delivers the drug to the outer layer of skin, ethosomes shows permeation through stratum corneum barrier. The main reason behind this deeper penetration and distribution of ethosomal drug in skin might be due to synergistic effect of combination of phospholipid and high concentration of ethanol [14].

3.1 Categories of ethosomes:

mainly there are 3 categories of ethosomes as above,

A. **Classical ethosomes:** These are modified form of classical liposomes which have better permeability. They mainly composed of phospholipid water and high concentration of ethanol up to 45%w/w. Classical ethosomes are more efficient than liposomes for transdermal delivery of drug because of their smaller size negative zeta potential and high entrapment efficiency causes better stability also [15,16]. The drugs with molecular weight 130.077 Da to 24 kDa can be encapsulated in this category [17].
B. **Binary ethosomes:** Binary ethosomes are prepared by adding the different alcohol in classical ethosomes such as isopropyl alcohol and propylene glycol [18].
C. **Transethosomes:** It is new generation of ethosomes. It is basically improved version of classical ethosomes with addition of either penetration enhancer or edge activator (surfactant) to the basic additives [19]. Drugs with low to high molecular weight can be entrapped with transethosomes. They are biodegradable and biocompatible in nature due to which it shows high entrapment efficiency.

3.2 Advantages:

Ethosomes have several advantages when compare to transdermal delivery system, following are the advantages [4],

i. It contains non-toxic excipients in its formulation. All the excipients are inert in nature.
ii. Delivery of large molecules like peptides and protein molecules through ethosomes is possible. It can entrap all types of drug molecules from hydrophilic to lipophilic to amphiphilic. From solid to liquid also.
iii. Ethosomes improve the permeability of drug through skin.
iv. It is simple method for formulation when compares to Phonophoresis, sonophoresis and iontophoresis. No any special equipment is required to manufacture with no complicated technical investments required.
v. The ethosomal system is passive non-invasive and available for immediate action.
vi. Ethosomes system is efficient to deliver a fluorescent probe to skin in terms of quantity and depth.
vii. Low risk profile.

viii. High patient compliance: As ethosomes administered is semisolid form such as gel or cream causes highly accepted by patient from pediatric to geriatrics. On the opposite side, iontophoresis, phonophoresis are complicated to use.

ix. Ethosomal drug delivery system can be widely used in cosmetic, pharmaceutical, pesticidal and veterinary industry.

3.3 Disadvantages:

i. Sometimes gives very poor yield.

ii. Absorption at percutaneous membrane is still depends on molecular size of drug.

iii. Skin irritation or dermatitis may be occurred in some patients because of penetration enhancer or due to ethanol or other excipients.

iv. Ethosomes with poor shells may clump together causes precipitation.

v. It may not adhere well to all types of skin.

vi. Drugs require higher blood levels can't administered. So only limited to potent drugs like such drugs which having daily dose 10 mg or less. [20-22]

4. Mechanism of ethosomes:

The main advantage of ethosomes over liposomes is it increases the permeability of the drug. Although the mechanism of the absorption of drug from ethosomes is not clear. The drug absorption may occur in following two phases:

A. Ethanol effect

B. Ethosomes effect

A. **Ethanol effect:** Ethanol is use to enhance the penetration of drug through the skin. Its penetration enhancing effect mechanism is well known. Ethanol penetrates through intercellular lipids and decrease the density of lipid multilayer of cell membrane and increases the fluidity of cell membrane lipids.

B. **Ethosomes effect:** Increased cell membrane lipid fluidity due to ethanol presents in ethosomes which increased permeability of skin. So, the ethosomes permeates through deep skin layers very easily, where it fused with skin lipids and releases the drugs into deep layer of skin [23].

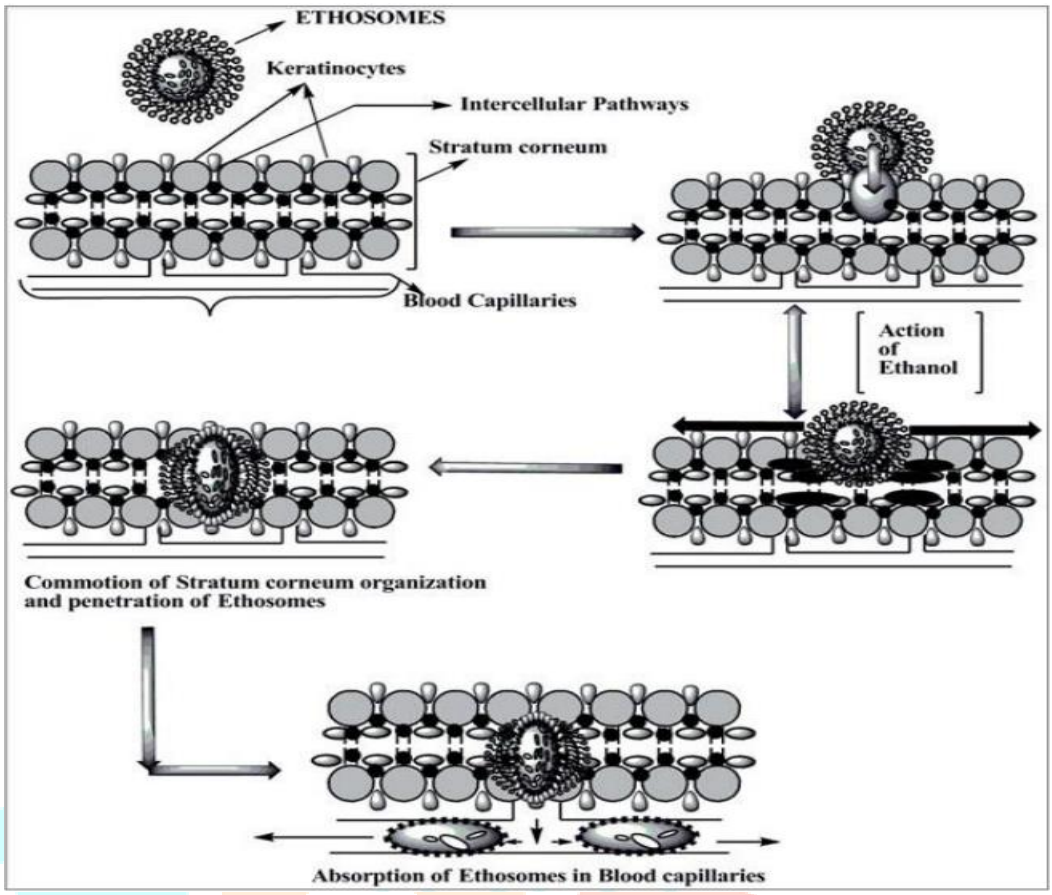

Fig. 2: Mechanism of action of ethosomes

5. Effect of High alcohol content:

Ethanol is an effective permeation enhancer and it is in high concentration ranging 20-25% in ethosomes. So, ethanol gives synergistic effect. The synergistic effect of high concentration of ethanol in ethosomes vesicles is the main reason for their better permeability through skin. The high concentration of ethanol in ethosomes may disrupt the skin lipid bilayer organization. When integrated to the vesicles to permeate through stratum corneum layer because of high ethanolic concentration, the ethosomal lipid membrane was packed less tightly than conventional vesicle. Ethosomes squeezes through small openings created in the disrupted stratum corneum layer. The vesicular nature of ethosomal formulation can be modified by changing the ratio of components and chemical structure of the phospholipids [24-26].

6. Factors affecting the properties of ethosomes:

I. Physicochemical properties of drugs: It may affect to the ethosomal properties like change in particle size, entrapment efficiency, zeta potential and its nature. According to Lodzki, trihexyphenidyl hydrochloride, diclofenac sodium, cromolyn sodium, and buspirone hydrochloride incorporated in the ethosomal system causes decreased ethosomal size. This is because of surface active properties of ethosomes. While according to Paolino, Paclitaxel incorporated in ethosomal formulation causes increased vesicle size [27,28].

II. Shumilov and Touitou described about the consigned frequently bare ethosomal system as pessimistic (-8.8 mV) and moved to certainty (7.16 mV) when of 30 mg buspirone hydrochloride incorporated in ethosomal system. An analogous determination was done with trihexyphenidyl hydrochloride 0.5%

w/w, where the vacuous ethosomes pessimistic charge (-4.5 mV) moved to an optimistic charge (4.8 mV). This effect was based upon the denseness of trihexyphenidyl hydrochloride added. Increasing the percentage of the drug to 1% and 3% w/w resulted in a correlative increase in ζ-potential values of 7.2 mV and 10.4 mV respectively [29, 30].

III. Physicochemical properties of formulation ingredients: these also affect to the properties of ethosomes similar to drugs. So, the additives used in formulation should be chemically and physically stable, inert, non-reactive to drug.

IV. Stirring time and temperature: although this may not affect more to the ethosomes but sometimes temperature with more than 40^0C can be affect the concentration of ethanol inside the ethosomes. while increased stirring time can cause disruption of ethosomal vesicles.

V. Sonication: excessive sonication can cause disruption of vesicles or may release of ethanol from vesicles.

7. Composition of ethosomes:

Ethosomes mainly consists of phospholipid ethanol and water in there composition alongside with drug [5]. The phospholipids varying in chemical structure includes phosphatidylcholine (PC), hydrogenated PC, phosphatidyl glycerol (PPG), Phosphatidylethanolamine(PE), phosphatidylinositol (PI), phosphatidylserine. Alcohol used in formulation of ethosomes is mainly ethanol for classical ethosomes but now the ethosomes are in advanced form like binary ethosomes where with ethanol another type of alcohol is also used like isopropyl alcohol propylene glycol. The non-aqueous phase is ranges from 22-70%. Cholesterol (0.1-1) also used to increase stability. Dyes or amphiphilic fluorescent probes like Rhodamine- 123, Rhodamine -B, D-289, fluorescence isothiocyanate (FITC), 6- Carboxy fluorescence often added to ethosomes for the characterization study of ethosomes [6, 31,32].

Table no. 1: Different additives employed in formulation of ethosomes:

Sr. No.	Class	Example	Use	Concentration
1	Phospholipid	Soya Phosphatidyl choline Egg phosphatidyl choline Dipalmitoyl phosphatidyl choline Distearyl phosphatidyl choline	Vesicles forming Components	0.5-10% w/w
2	Polyglycol	Propylene glycol Transcutol RTM	Skin permeation enhancer	22-70% w/w
3	Alcohol	Ethanol Isopropyl Alcohol	Penetration enhancer To provide softness to the membrane of vesicles.	
4	Cholesterol	Cholesterol	To stabilise the vesicles	0.1-1% w/w
5	Dye	Rhodamine 123 Rhodamine red fluorescence 6-Carboxy fluorescence		-
6	Vehicle	Water	Solvent. To dilute the final formulation.	Q.S.
7	Others	Diethyl phosphate	For the prevention of aggregation of vesicles	-

8. Method of preparation of ethosomes:

The procedure for preparation of ethosomes is very simple as compare to other nanoparticle preparations. There is no any costly instrument required for ethosome preparation. Ethosomes are generally prepared by following methods: [33-35]

I. **Cold method:** It is most widely used method for synthesis of ethosomes. In cold method phospholipids and drug dissolved in ethanol and glycol at room temperature. Sometimes along with phospholipids other lipids like cholesterol also added to stabilize the preparations. This mixture is stirred at 700 rpm at 30^0c. after 5 minutes distilled water previously heated at 30^0C is added to ethanolic solution with syringe at 700 rpm. Aloe to stir for 30 minutes. Vesicle size of ethosomes can be decreased with the help of extrusion or sonication [36]. While performing the method the vessel containing ethanol should be well covered to avoid the evaporation of ethanol. The final product should be stored in refrigerator.

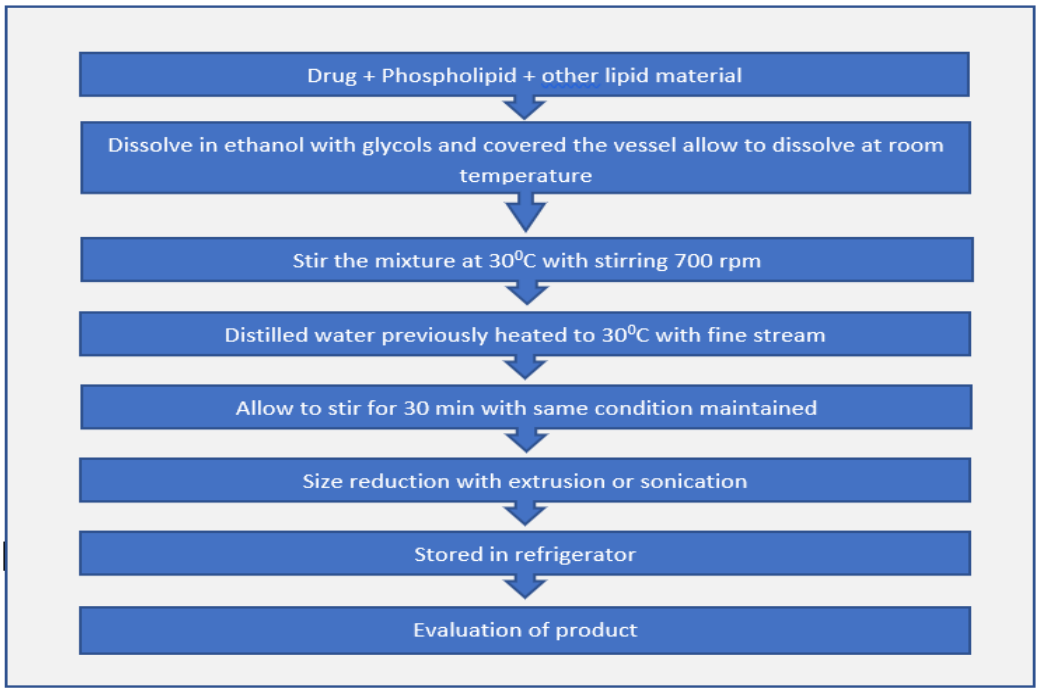

Fig. 3: Preparation of ethosomes by cold method

II. Hot method:

In this method, Phospholipids are first dispersed in distilled water and heated to 40^{0}C with constant stirring. Simultaneously ethanol with propylene glycol is heated to 40^{0}C. Drug is either dissolved in aqueous phase or organic phase according to its solubility. Then organic phase is added slowly to aqueous phase with constant stirring 700 rpm for 30 minutes at 40^{0}C. The final product is sonicated to reduce the particle size **[33,37,38].**

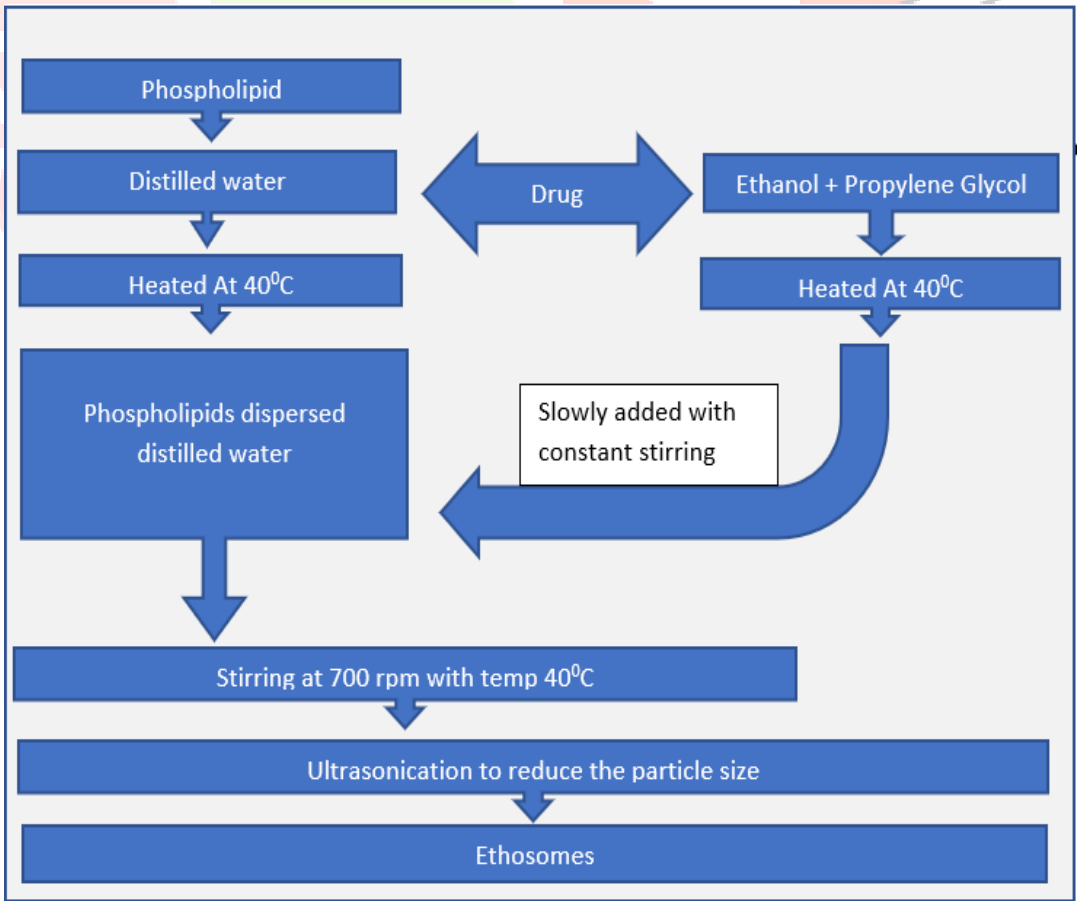

Fig. 4: Hot Method for ethosomes

III. **Thin film hydration method:** here, drug and phospholipids dissolved in chloroform: methanol in 3:1 ratio and kept in round bottom flask and then evaporated in rotary evaporator above lipid transition temperature i.e. above 60^0C to evaporate complete methanol and chloroform to form a thin film in RBF. Then it is hydrated with phosphate buffers saline with pH 7.4 containing ethanol. Then the formulation is sonicated for 5 minutes to reduce the particle size to give ethosomes. Then stored in refrigerator. This method is similar to that of Phytosomes but here only slight change in buffer with ethanol [39].

IV. **Classic method:** In this method, Drug and phospholipid are dissolved in ethanol and heated to 30°C±1°C in a water bath. Double distilled water is added in a fine stream with syringe to the lipid mixture, with constant stirring at 700rpm, in a closed vessel to avoid evaporation of ethanol. This vesicle suspension is then homogenized by passing through a polycarbonate membrane using a hand extruder for three cycles [40].

9. Methods of characterization of ethosome

i. **Shape and size of vesicle**:

The shape of the vesicle determined by visualization using SEM (Scanning Electron Microscopy) & TEM (Transmission Electron Microscopy) [41] and size of vesicles can be determined by Photon correlation spectroscopy and Dynamic light scattering method. Ethosomes generally have vesicular size ranging from tens of nanometre to micrometre.

ii. **Zeta potential:**

By using zeta meter for determination of the particle surface charge which is used to predict the stability of ethosomes.

iii. **Bilayer Configuration:**

Ethosomes entrap lipophilic as well as hydrophilic drug depend on their high degree of lamellarity so the optimum bilayer formation study should be conducted. It can be performed with the help of NMR (Nuclear Magnetic Resonance).

iv. **Entrapment Efficiency:**

This can be done by two methods,

a. **Ultracentrifugation**:

It's a two-step method in which first step is to kept the ethosomal preparation overnight and subjected to ultracentrifugation for a particular period of time. In second step, pure drug is evaluated with help of highly developed method such as HPLC (High-Performance Liquid Chromatography) or UV spectroscopy.

b. **Dialysis:**

Polymers like e.g. cellulose acetate are used for preparation of dialysis bags in which the calculated amount of the drug-loaded vesicles or free drug in aqueous solution are loaded which then transferred to 500 ml of phosphate buffer pH 7.0. The mediums are stirred with help of magnetic

stirrer. The samples is withdrawn within fixed time interval from the buffer medium and replaced with equal volumes of 7.0 phosphate buffer saline solution to maintain the sink conditions. Entrapment efficiency of both methods can then be finally calculated using [2,42,43].

$$EE = \frac{Dt - Ds}{Ds} \times 100$$

Where,

EE: Entrapment Efficiency

Dt: Theoretical amount of drug

Ds: Amount of drug detected in supernatant layer

v. **Surface tension measurement:**

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

vi. **Transition temperature:**

The transition temperature of ethosomes can be measured by DSC (Differential Scanning Calorimetry) at 10˚C per min in an aluminium pan under a constant nitrogen stream [44].

vii. **Skin permeation studies:** Confocal laser scanning microscopy (CLSM) method used to determination of the depth of penetration from Ethosomes. The ethosomes shows significantly higher skin deposition because of combination of ethanol and phospholipid thus providing a mode for dermal and transdermal delivery [45].

viii. **In vitro drug release study and Drug deposition study:** In vitro drug release study and drug deposition of ethosomal preparation can be performed with the help of Franz diffusion cell with artificial or biological membrane [45].

ix. **Stability studies** Ethosomes ability to retain the drug (i.e., drug-retentive behaviour) can be checked by keeping the preparations at varying temperatures at 25 ± 2°C (room temperature, RT), 37 ± 2°C and 45 ± 2°C for different time interval (1, 20, 40, 60, 80 and 120 days). The ethosomes should kept in sealed vials (10 ml capacity) after flushing with nitrogen [45].

10.Therapeutics application of Ethosomes:

Ethosomes can be used for various purposes for drug delivery system. Ethosomes are mainly used instead of liposomes. Transdermal route is preferred mostly for ethosomes. Ethosomes can be used for the transdermal delivery of hydrophilic and with low or no permeability of drugs through the skin. Various drugs have been formulated with ethosomal carrier [46,47].

I. **Pilosebaceous targeting:**

Sebaceous glands and hair follicles are being recognized as potentially significant routes in the percutaneous drug delivery. Many studies also focusing on exploring the follicles to transport the systemic drug delivery. Minoxidil is one of the lipid-soluble drug used topically on the scalp for the treatment of baldness by pilosebaceous delivery. Interest in pilosebaceous units has been directed

towards their use as depots preparations for localized therapy, mainly for the treatment of follicle-related disorders such as acne or alopecia [48].

II. Transcellular Delivery

Touitou et al. [44] demonstrated in their study about intracellular uptake of bacitracin, DNA and erythromycin using CLSM and FACS techniques in different cell lines. Anti-HIV drug zidovudine and lamivudine have best cellular uptake in MT-2 cell line from ethosomes as compared to the other marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

III. Delivery of problematic drug molecules:

Oral delivery of large biogenic molecules like peptides or proteins and insulin is very difficult due to their complete degradation in the GI tract. hence transdermal delivery is a better option. But conventional transdermal formulation of peptides or protein and insulin has very poor permeation. Formulating these above molecules into ethosomes significantly increase their permeability and also therapeutic efficacy (49).

IV. Delivery of antarthritic drug:

Drugs used in arthritis treatment are mainly associated with problems like low bioavailability, GIT degradation, first pass metabolism etc. To overcome above problems ethosomal formulation of antarthritic drugs can be a best alternative as it significantly increases skin permeation, accumulation and biological activity also (50)

V. Delivery of HIV drugs:

An effective antiretroviral therapy is required on a long-term basis which can also responsible for strong side effects. Adequate zero order delivery of zidovudine, Lamivudine a antiviral agent is required to maintain expected anti-AIDS effect (51, 52).

VI. Antiviral drugs:

Conventional topical preparation of acyclovir used as antiviral drug in treatment of herpes labials show low therapeutic efficiency because of the poor permeation through skin as replication of virus take places at the basal dermis. Acyclovir ethosomes show high therapeutic efficiency and higher percentage of abortive lesions.

VII. Cosmeceutical Applications of Ethosomes:

The advantage for formulating ethosomes in cosmetic industry is it enhances the stability of the cosmetic chemicals and decrease skin irritation problem arises from irritating cosmetic chemicals, also improves transdermal permeability, specifically in the elastic forms. However, the compositions and sizes of the vesicles of ethosomes are the main factors to be considered while formulation [53].

VIII. Marketed Product of Ethosomes: In 2000, the ethosomes technology began to Commercialize. Following are some of the market products of ethosomes mentioned in (Table 3).

Table No. 2: Marketed Products of ethosomal drug delivery system:

Sr. No.	Name of product	Manufacturer	Uses
1.	Nanominox	Sinere, Germany	First minoxidil containing product, which uses ethosomes. Contains 4% Minoxidil is a hair growth promoter that must be metabolized by sulfation to the active compound.
2.	Noicellex	Novel Therapeutic Technologies, Israel	Topical anti-cellulite cream
3.	Supravir cream	Trima, Israel	For the treatment of herpes virus.
4.	Decorin cream	Genome Cosmetics, Pennsylvania, US	Anti-aging cream. Used to treat, repair and delay the visible aging signs of skin such as wrinkle lines, sagging, age spots, dark spot, dark circles, loss of elasticity, and hyper pigmentation.
5.	Cellutight EF	Hampden Health, USA	Topical cellulite cream. It has powerful combination of ingredients to increase metabolism and break down fat.
6.	Skin genuity	Physonics, Nottingham, UK	Powerful anti-cellulite.

11.Patented and marketed formulation of ethosome:

Ethosomes were first invented and patented by Prof. E. Touitou and her students from Department of Pharmaceutics (Hebrew University School of Pharmacy) **[31,32].** Novel Therapeutic Technologies Inc (NTT) of Hebrew University have brought a number of products to the market based on ethosomes.

A. **Nanominox©** containing minoxidil is used as hair tonic to enhance hair growth. It is marketed by Sinere.
B. **Noicellex TM**: It is anti – cellulite formulation of ethosome is currently marketed in Japan.
C. **Lipoduction TM:** This formulation is used as anti-cellulite contains mainly pure grape seed extracts (antioxidant) is marketed in USA.
D. **Physonics:** It is anti – cellulite gel Skin Genuity in London.

12.Future Prospects:

- Introduction of ethosomes has given the wide scope in vesicular research for transdermal drug delivery.
- Ethosomes will allow better control over drug release *in vivo* with improves permeation allowing physician to make the therapy more effective and easier.
- It increases the permeability of drug so the drugs with permeability problem especially herbal drugs which having large molecular size and molecular weight.
- Ethosome offers non-invasive delivery of small, medium and large sized drug molecules.
- Ethosomal formulations have promising future in effective dermal/transdermal delivery of bioactive agents.
- Preparation is also simple as the method and no heavy or highly costed instrument is required.

- So ethosomes have very wide scope and future in pharmaceutical industry, nutraceuticals industry, cosmetics industry, pesticidal industry and veterinary industry.

13.Conclusion:

Currently ethosomes have a highly attractive raised area or a diverse range of applications. The research works shows that ethosomal systems have immense potential to alter poorly soluble, poorly absorbed, and having poor penetrability active material into capable delivery drugs. Ethosomes have potential carriers to transport drugs across the scalp for treating androgenic alopecia and male pattern hair loss. Drugs like finasteride and minoxidil may be explored for topical applications by using ethosome to overcome the conventional dosage form side effect. Herbal ethosomes is a new area in vesicular research to transport the herbal molecules inside the body. Even phytoconstituents having promising therapeutic effects, most of them are fail to achieve bioavailability because of their poor absorption. Large molecular sizes and low lipid solubilities are the main factors causing poor absorption of phytoconstituents results in low bioavailability. So ethosomes has a promising future in herbal drug technology to increase penetrability and bioavailability of phytoconstituents. Ethosomes systems offers a superior prospect for the non-invasive delivery of small, medium and large-sized drug molecules, provides good patient compliance and low-cost treatment. Ethosomes generates new challenges and opportunities for researchers for the development of novel therapies. Further research will allow better control over drug release *in vivo* and long-term safety data, allowing the therapy more effective and less toxic.

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