ABSTRACT

Background: In the past many years, there have been many studies that are considering the nanoparticles as the carriers of the drugs for improving the patient compliance. One such approach of delivery of the drug is through the ethosomes. Objectives: This review aims to describe the vesicular approach of ethosomes that had been used to overcome the barrier for the transdermal delivery of drug and also describes brief composition, method of preparation, characterization tests, mechanism of penetration of ethosomes, as well as highlighted various studies of ethosomes in medicine. Methodology: Relevant data was searched using various databases like Google Scholar, PubMed, SpringerLink and Scopus. Results: Ethosomes are the vesicular shaped structures comprising majorly of phospholipids and ethanol. Due to their size in nanometers and presence of increased amount of the ethanol in its structure, it had shown very high permeation of the drug when given through the transdermal route or topically. There are basically two methods for the formulation of the ethosomes that includes the hot method and cold method. Furthermore, recently more two methods have also been developed for formulating ethosomes including classical mechanical dispersion method and transmembrane pH gradient method. After formulation of ethosomes, many characterization tests can be performed on ethosomes such as pH, particle size, zeta potential, in vitro permeation studies, microscopic evaluation and stability studies. Conclusion: Many more studies are needed to enhance these novel drug delivery systems and other vesicular systems such as transferosomes and invasomes for better therapeutic output and ultimately increasing the patient compliance.

Keywords: Nanoparticles, Compliance, Transferosomes, Ethosomes, Transdermal, Transmembrane, Permeation.
INTRODUCTION

Advancements in the approaches of drug delivery are occurring at a lot quicker pace as comparatively with the most recent twenty years. Improved patient compliance and viability are the main objectives of the new drug delivery systems. A more extreme methodology has been to investigate the new interfaces on the body for the administration of the medications. One such methodology, the transdermal delivery of drug system, utilizes skin of human as a portion of the body of entering for the systemic delivery of drug molecule (1). The application of novel approaches can also improve the efficacy of herbal cosmetic formulations on the human body (2).

In the past years, the process of the nano ionization of drugs had attracted much more attention than the other medications (3). The nanoparticles are defined as colloidal systems with size of the particles varying from the 1 nm - 1000 nm (4, 5). The nanoparticles with mean of the particle size higher than the 100 nm standard had been also reported in the literature, that includes the nanosized curcuminoids (6), paclitaxel (7) and praziquantel (8) that had the mean particle size of about 450, 147.7 and likely to be higher than the 200 nm respectively. Additionally, the nanoparticles can also be defined as the submicron (<1 ml) colloidal systems (9). Recent advancement in the transdermal drug delivery system (TDDS) is having much more benefits, particularly for the drugs that had a very poor permeation in the stratum corneum of the skin (10). NSAIDs drugs are the most widely used as anti-inflammatory, analgesic as well as anti-pyretic. Anti-inflammatory effect of the NSAIDs is due to cyclooxygenases (COX-II) inhibition and thus decrease the prostaglandin formation that increases the serious side effects or adverse effects mainly on stomach by the means of systemic administration (11). Therefore, some NSAIDs could be administered as transdermal for accomplishing systemic or the local effect as the substitute option for parenteral and oral administration of the medication. Some new formulation methods had been formed for the delivery of the NSAIDs (12).

TDDS exhibited auspicious result conversely, by the oral drug delivery as this kill GIT deterrants as well as hepatic metabolism of drugs yet main disadvantage of transdermal drug delivery is that it encounters obstruction properties of the layer of the skin i.e., Stratum corneum, for instance lipophilic medicines having sub-atomic weight < 500 Da can go through it. To work on the entrance of medications through the skin, diverse different means had been examined, that includes the utilization of compound and the permeation enhancers, for instance, sonophoresis, iontophoresis, etc. ethosomes, transferosomes, liposomes and the niosomes, are also had been considered to increase the permeability of medicine by layer stratum corneum. Penetration enhancers can be utilized to achieve this, with the objective that the medications can get through the skin with no issue. Ethosomes can penetrate through the skin layers more rapidly and have in a general sense higher transdermal results conversely, with liposomes (13).

Transdermal drug delivery has additionally upper hands over hypodermic infusions that can be painful, produces risky medical waste as well as can have danger of infection transmission by the needle re-use, particularly in advanced countries. Additionally, transdermal delivery is painless and could easily self-administer. It can provide delivery of the drug to a longer period of time (as long as weeks). Therefore it can also increase patient compliance and these systems are mostly inexpensive (14). Bioavailability overall could be easily controlled and measured by this dosage form of the drug. Here the bioavailability of a medication differs a hundred or multiple times with the dosage form and as a resultant might influence the safety and efficacy of the medication. Transdermal therapeutic systems and Drug delivery systems are progressed types of innovation for the control of bioavailability through the dosage form (15).

TDDS could be viable for the drugs which go through broad hepatic metabolism, and were mainly unstable in GIT lot, as well as additionally causes extreme adverse effects when provided as oral medication. These drug delivery systems likewise give controlled drug delivery, decreased dosing recurrence and increases the compliance of patient (16). The major transference mechanisms through which medications crosses intact skin layers are as yet not explained in spite of numerous long years of studies and examination. The most possible routes of administration involve the
trans epidermal pathway (through horny layer either intercellularly or can be intracellularly) or through the follicles of hair and the sweat glands which comprises of the appendageal way. However, some disadvantages of the system incorporate:

1) Medications which require the high blood levels could not be administered by means of this.
2) Drug or the drug formulation might cause the skin sensitization as well as irritation.
3) The adhesive might not stick well to a wide range of the skin.
4) Can be uncomfortable to wear (17).

Topical route of delivery system is another technique that empowers the medication to reach to the systemic circulation. In pharmaceutical point of view topical drug delivery offer benefits contrasted and different routes of administration, that includes the aversion of the hepatic metabolism, less administration recurrence, lesser variations in the plasma drug profile, as well as increased patient consistence (18). Topical delivery could be recognized as the use of a medication that contains the formulation of the skin directly to deal with skin problems like skin inflammation or the cutaneous indications of a general infection like psoriasis with the aim of containing the therapeutic or other impact of the medication to the outer layer of the skin or inside the skin. Topical drug delivery offers the benefits of simplicity of delivery, an increased compliance, expanded consistence just as the aversion of first-pass metabolism. Disadvantages are the absence of, or decreased rates of the absorption and cosmetic considerations (19).

Topical drug delivery systems are made of different ingredients, for example, an ointment base, emulsifying agent, buffering agent, vehicle, thickening agent, antioxidant, additives, and permeation enhancer. Among the ingredients, the penetration enhancer expands the drug delivery by advancing the diffusion, partitioning, or solubility of an active ingredient through the layer corneum (20). In the course of the last many years, the basic treatment of ailment had been refined by administering the drug to the human body through many different routes to be specific sublingual, oral, parental, rectal and so forth. Topical drug delivery is mainly be used whereby the other of all the systems of drug administration are not successful or can be in local skin infection just like the fungal infections. Topical drug delivery could be characterized as use of the medication that contains the formulation applied to the skin for the treatment of the cutaneous disorder (21).

Skin

The skin goes about as a strong boundary between entry of any substance and the environment changing attack of the microbes. The major impediment is primarily restricted in between the layer corneum and includes the protein enriched cells that are the corneocytes with cornified envelope as well as the parts of the cytoskeletal, similarly as the corneodesmosomes and the other lipid enriched intercellular regions as shown in Figure 1 (22).

Skin is most wide organ and is readily available organs of human body system; simply a modest quantity of a millimeter of tissue isolates the skin surface. A perception of the nature and base of the deterrent properties of skin, and of the physicochemical characteristics of substances which choose their ability to penetrate through the skin and enter the stream, would have incredible incentive to ecologists and toxicologists, stressed over the dangers of skin opening to air and to water toxins and presently most of the doctors and pharmacologists are keen on the use of skin as a course of section of meds for the treatment of or dermatological and a lot more infections (23).

Figure 1. Anatomy of skin (24)

Ethosomes

Elka Touitou, the inventor and having authorization of numerous patents that also includes the ethosomes, which is a perceived nanocarrier for improved dermal and
transdermal delivery (25). Ethosomes are a fascinating and an innovative vesicular drug delivery system that have showed up in the fields of drug innovation and drug delivery as of late (26). The delicate vesicles represent the new vesicular transporter to improve delivery of the drug through the skin. The ethosome is a creative vesicular framework that shows alluring features related with the large deformability. The ethosomes could carry active agents through layer corneum into more profound layers of skin more viably than customary liposomes (26). Size of ethosomes vesicles could be adjusted by several nanometers to microns (27). Because of their size (roughly 150–200 nm) and the high deformability, they are additionally alluded to as the elastic nanovesicles. These vesicles are equipped for entering by the pores of size smaller than its own size. These type of vesicles could easily be applied to the skin in the form of ointment or a gel (28). They give sustained delivery of the drug as well as could go about as a transporter for both hydrophobic and hydrophilic drugs (29).

This system had great capability to penetrate through human skin because of its large elasticity properties, that had a huge ramification for plan of transporter system that can be applied topically for both systemic as well as local delivery of lipophilic and hydrophilic medications (30).

In 2000 Touitou and Dayan had compared the classic liposomal system with ethosomal delivery of the trihexyphenidyl HCl, later they had found that ethosomes significantly upgraded skin pervasion of trihexyphenidyl HCl in vitro (31). Then later transdermal drug delivery system for cannabinoids with utilizing ethosomal transporter were likewise formulated by Lodzki and associates (32). In 2004, Touitou and Godin researched intracellular and dermal delivery of the bacitracin from the ethosomes (33). Later that Elasayed et al. (2006) concentrated on mechanism by then ethosomes could further develop skin delivery of ketotifen in the nonocclusive conditions (26, 34). Ethosomal systems are the vesicles comprising basically of phospholipids, water, and a high amount of ethanol (Figure 2). Phospholipids can be utilized mostly at 0.5%–10% concentration range, and are acquired from natural synthetic and semisynthetic sources like soybean and egg. Some common examples of phospholipids incorporate phosphatidylethanolamine, phosphatidylinositol, phosphatidylcholine, and hydrogenated phosphatidylcholine (35). Ethanol could mostly be used at a concentration of 20%–45%, that functions as an proficient skin enhancer (36). Ethanol also played same role as that played by surfactants in the case of the transferosomes through disorganizing lipid layer (37).

![Figure 2. Structural composition of ethosomes (38)](image)

By virtue of their unprecedented design, ethosomes could epitomize and pass on through the skin, extraordinarily lipophilic atoms, for instance, minoxidil, testosterone, and cannabinoids, similarly as cationic drugs, for instance, trihexyphenidyl and propranolol (38). An effective assessment of the effect of plan on vesicular size demonstrated that additions in the percent of lipid in this will help in achieving the greater vesicles, while increasing the percent of ethanol at a comparable lipid center decay the size of the ethosomes (25).

**Ethosomal drug delivery through the skin**

The shallowest layer of the skin is the epidermis and is made out from the separated keratinized squamous epithelium cells which does differs in the thickness in many parts of the body. This is the thickest and consists of the flexible fibers. Skin shapes a moderately water covering layer which ensures more profound and much more fragile structures. The Blood vessels are mainly disseminated abundantly underneath skin (39).

Drug penetration across the skin is ordinarily through diffusion and obeys the Fick's law of dispersion. In any case, on the off chance that the medication is especially lipophilic and
furthermore utilized in the most suitable skin, the physicochemical intuitiveness of the medicine with the skin might accept a critical part for the drug transport across the skin (40). A numerical model of layer corneum as a two phase protein lipid heterogeneous film (in which the lipid stage is consistent) associates the vulnerability of the film to a specific penetrant with the water dissolvability of the penetrant and with its lipid-protein fragment coefficient. Likely assessed permeabilities of human skin to a combination of medications have been found to conform to this model. The incredibly low penetrability of skin to the most micro molecules appears to arise out of the very low diffusivity of such particles in the intercellular lipid stage (23).

In 2000, Touitou et al. delineated mechanism of the ethosomes in which they had involved ethanol as the penetration enhancer (Figure 3). These principally acts through the synergistic activity of the ethanol, vesicular framework just as the lipids found in skin (26). Ethanol additionally diminishes the glass transition temperature of lipids of the skin as well as of the vesicles (41).

**CATEGORIES OF ETHOSOMES**

Following are some major classifications of ethosomes.

**Classical ethosomes**

These are the altered classical liposomes that show much better skin pervasion. These are made out of phospholipids, a relatively higher concentration of the ethanol that is up to the 45% w/w and water. The classical ethosomes are viewed as astounding over the liposomes for transdermal delivery of drug by virtue of its smaller vesicular size, negative zeta potential and as well as higher entrapment efficiency (43).

**Binary ethosomes**

These were created by the addition of an alternate sort of liquor to the traditional ethosomes. Most usually utilized alcohols includes, isopropyl alcohol as well as the propylene glycol (44).

**Transethosomes**

These are known to be the new and most advanced ethosomal system. This comprises of fundamental part of classical ethosomes and an extra part that is either an edge activator or a penetration enhancer for example, a surfactant (45).

The structural representation of liposomes and other different types of ethosomes is illustrated in Figure 4.

**Advantages of drug delivery through ethosomes**

Various advantages have been offered by using the skin delivery of the drug, for example the sustained release from drug, enhanced patient compliance as well as much more access to the systemic or local target sites (46). Many strategies had now been formed to minimize poor skin permeation of the nanocarriers that includes the liposomes.

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**Figure 3. Schematic representation of ethosomes skin permeation mechanism:**

1) the ethanol content of ethosomes disturbs the organization of the SC lipids;
2) ethanol confers ethosomes increased fluidity and flexibility;
3) ethosomes display enhanced penetration through deeper skin layers (42)

**Figure 4. Structural representation of liposomes and different types of ethosomes (42)**
Certainly, now in much of the cases these ethosomes had shown some better and enhanced delivery through skin, when these were compared with the classical liposomes (47). Key characteristic that mainly separates the ethosomes from all other nanocarriers was its capability to transport the active ingredients either they are lipophilic or hydrophilic into the much deeper layers of the skin, and their enhanced application for both the non-occlusive as well as occlusive conditions (48-51). Frequently, the active ingredients that are hydrophilic remains in aqueous core, whereas the lipophilic as well as the amphiphilic active ingredients basically interacts widely with lipid bilayer of the vesicle (52, 53). Furthermore, the ethosomes shows some deformable character, much large entrapment efficiency, more stability at room temperature, and much more biocompatibility with the Stratum corneum (54). Incorporation of the larger concentrations of ethanol in the ethosomes shows the main features toward delivery of skin of the active ingredients, the ethosomes have now became much more elastic as well as deformable, that increases its permeation through the skin layer (55-57). Moreover, ethanol minimizes phase transition temperature of stratum corneum bilayers of lipid, thus it increases their permeability as well as fluidity, and it allows the embolism of ethosomes with stratum corneum (58, 59). Lastly, ethanol imparts negative charge to the ethosomes, that leads to the decrease in size of the vesicles, and thus increases the bioavailability of the active ingredients (59).

**Drugs whose ethosomes were formulated**

Various drugs that have been incorporated in ethosomes along with their results have been mentioned in Table 1.

**METHOD OF PREPARATION**

Recent studies have shown two general methods for the preparation of ethosomes that are discussed as follows:

**Hot method**

In this procedure, scatter the phospholipid in water by warming in a water bath at 400 °C till a colloidal arrangement is acquired. In a different vessel appropriately mix the propylene glycol and ethanol and hot up to 400 °C. Then add the phase which is organic into the watery phase. Now, dissolve down the active drug in ethanol or water relying upon its solvency. The size of the vesicle of ethosomes can be reduced to the required extent utilizing extrusion method or the probe sonication (83). The drug is mixed in ethanol or water relying upon its hydrophobic /hydrophilic properties (36). The vesicles when formulated, are then extruded or sonicated to achieve the desired size (84).

**Cold method**

The most common and easy method for the formulation of the ethosomes is the cold method. At room temperature, dissolve the phospholipid, drug and remaining other lipid materials in ethanol in a covered vessel with the strong vigorous stirring. Then add propylene glycol or other polyol during stirring process. The mixture is heated up to 300 C in water bath. Then, water is heated up to 300 C in the separate vessel and then is added to mixture and finally is stirred it for in a vessel that should be covered for 5 minutes. Size of the vesicle of the ethosomal formulation could be reduced to the required extent by using extrusion or sonication method. In the end, formulation should be stored under in the refrigeration (74, 85).

**Classic mechanical-dispersion method**

By mixing the soya PC in mixture of methanol: chloroform (1:3) in the round bottom flask, ethosomes can be prepared. Organic solvents area then separated by the help of rotary vacuum evaporator with transition temperature of lipid of about 60°C, till some single thin layer of lipid film was shaped on the flask wall. Remaining amount of the solvent is then removed from deposited lipid film layer by leaving contents under the vacuum overnight. This is then followed by the hydration with some different concentrations of hydroethanolic solution, that contains the main active ingredient by rotation of the flask with some suitable temperature and conditions (81, 86, 87).
### Table 1. Studies on ethosomes

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug</th>
<th>Drug Category</th>
<th>Study Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Meloxicam</td>
<td>Anti-inflammatory (NSAID)</td>
<td>Compared to rigid liposomes these ethosomes are 3.77 X more permeated and penetrates through the skin, Entrapment efficiency: 78.25%.</td>
<td>(60)</td>
</tr>
<tr>
<td>2.</td>
<td>Cetirizine</td>
<td>Anti-histaminic</td>
<td>Ethosomal vesicles when compared to the other formulations, Ex-vivo penetration studies through the skin of the mice showed much larger and greater permeation flux of 16.300 ± 0.300 mg/h/cm2 and also the skin retention of 20.686 ± 0.517 mg/cm2</td>
<td>(61)</td>
</tr>
<tr>
<td>3.</td>
<td>Lamivudine</td>
<td>Anti-viral</td>
<td>Lamivudine ethosomes showed 25 times higher transdermal flux than the lamivudine solution. Also, it had shown higher intracellular uptake of the ethosomes (85.7%) when compared with the drug solution (24.9%)</td>
<td>(62)</td>
</tr>
<tr>
<td>4.</td>
<td>Vancomycin</td>
<td>Antibiotic</td>
<td>This type of the novel drug delivery system had shown to deliver drug above the MIC (Minimum inhibitory concentration)</td>
<td>(63)</td>
</tr>
<tr>
<td>5.</td>
<td>5-Fluorouracil</td>
<td>Anti-cancer and Laryngotracheal Stenosis</td>
<td>Entrapment efficiency: 12.25%. The accumulation of the 5-Fluorouracil in the skin and in the HS after 24 hours was: E-Scar &gt; H-Scar &gt; E-Skin &gt; H skin in the rabbits.</td>
<td>(64)</td>
</tr>
<tr>
<td>6.</td>
<td>Aceclofenac</td>
<td>NSAID</td>
<td>Drug permeation through the membrane: 0.26 to 0.49 mg/cm2. Entrapment efficiency: 91.06%</td>
<td>(65)</td>
</tr>
<tr>
<td>7.</td>
<td>Tretinoin</td>
<td>Anti-acne, Anti-neoplastic and Anti psoriatic</td>
<td>This ethosomal formulation enhances the photostability, anti-psoriatic activity, biocompatibility and the percutaneous absorption.</td>
<td>(66)</td>
</tr>
<tr>
<td>8.</td>
<td>Acyclovir</td>
<td>Anti-viral</td>
<td>The entrapment efficiency with solid lipid nanoparticles was recorded as only 53% with the ethosomes it was observed as 94%.</td>
<td>(67)</td>
</tr>
<tr>
<td>9.</td>
<td>Finasteride</td>
<td>Steroids (5-alpha reductase inhibitor)</td>
<td>Transdermal fluxes from ethosomes were 2.6, 3.2 and 7.4 times higher as compared to the hydroethanolic solution, conventional liposomes and aqueous solution, respectively.</td>
<td>(68)</td>
</tr>
<tr>
<td>10.</td>
<td>Testosterone</td>
<td>Growth Hormone</td>
<td>Permeation of skin through the patch of the ethosome was observed to be about 30 times much more than the testoderm™ patch in the rabbits.</td>
<td>(51)</td>
</tr>
<tr>
<td>11.</td>
<td>Valsartan</td>
<td>Anti-hypertensive</td>
<td>34.11% decrease in BP throughout the in-vivo study in rat. This effect lasts for about 48 hours.</td>
<td>(69)</td>
</tr>
<tr>
<td>12.</td>
<td>Ciclopirox-olamine</td>
<td>Antifungal drugs</td>
<td>EE of the ethosomes was 72.81% while in liposomes was observed to be 32.8%.</td>
<td>(70)</td>
</tr>
<tr>
<td>13.</td>
<td>Clotrimazole</td>
<td>Anti-fungal</td>
<td>Largest entrapment efficiency in the ethosomes i.e., 68.7% when compared to the ultra-deformable liposomes i.e., 55.51% along with the high flux (56.25 ± 5.49 µg/cm2/h)</td>
<td>(71)</td>
</tr>
<tr>
<td>14.</td>
<td>Azelaic acid</td>
<td>Anti-keratinizing agent</td>
<td>Ethosomal formulation release rate was observed to be more than that from liposomes.</td>
<td>(72)</td>
</tr>
<tr>
<td>15.</td>
<td>Ketotifen</td>
<td>NSAID</td>
<td>When compared with other topical formulations, Ethosomes had shown to deliver the entrapped drug into deeper skin.</td>
<td>(37)</td>
</tr>
<tr>
<td>16.</td>
<td>Diclofenac</td>
<td>NSAID</td>
<td>In vivo studies had shown that optimized</td>
<td>(73)</td>
</tr>
</tbody>
</table>
potassium ethosomal hydrogel exhibited enhanced anti-inflammatory activity compared with the plain drug hydrogel formulations and liposomal formulations. Optimized formulation had ethanol concentration of 22.9% and PC:CH ratio of 88.4:11.6, with vesicle size of 144 ± 5 nm, elasticity of 2.48 ± 0.75, zeta potential of -23.0 ± 3.76 mV, entrapment efficiency of 71 ± 4% and permeation flux was 12.9 ± 1.0 μg/h cm².

17. Fluconazole Antifungal

Entrapment efficiency was observed to be 82.68% for ethosomes and 68.22% for liposomes. Drug diffusion rate also found to be twice when compared with the liposomal formulation.

18. Ropivacaine Local anesthetic

Ex vivo permeation through mice skin was 349.0 ± 11.5 μg cm² at 12 h

19. Salbutamol sulfate Sympathomimetic (beta2-adrenergic agonist)

Bronchodilation was increased when delivered by the transdermal ethosome as compared to the conventional vesicles.

20. Apigenin Anti-inflammatory flavonoid

The Apigenin encapsulation was much increased by the increase in phospholipid conc., larger deposition through the skin was detected with ethosomes that was 0.188 μg/cm² when compared with liposomes that was about 0.109 μg/cm² after the 12 hours.

21. Repaglinide Ani-diabetic

Ex-vivo skin permeation was 64-97% while the Entrapment efficiency was 75-92%

22. Erythromycin Macrolides

No subdermal healing; Deep dermal abscesses. Ethosomal group: No bacterial growth and normal skin structure. Hydroethanolic erythromycin solution group: the efficacy of formulation of ethosome was found to be the same as of systemic delivery in S. aureus infected mice.

23. Melatonin Neurohormone

This study had shown enhanced transdermal flux i.e., 59.2+/−1.22 μg/cm²/h as well as decreased lag time, i.e., 0.9 h across the human cadaver skin, Entrapment efficiency: 70.71% +/-1.4%

24. Minoxidil Hair growth promoter

This study had shown 5 folds increased accumulation in the mice skin when compared with the ethanolic solution of drug.

25. Ammonium glycyrrhizinate Anti-inflammatory drug

Entrapment efficiency was observed to be 81.7%.

26. 5-aminolevulinic acid Photo-dynamic therapy

The skin production of protoporphyrin IX was increased 11-15 folds when associated with the control formulation.
**pH-Gradient method (Transmembrane)**

This methodology comprises of the two individual steps: first step includes preparation of the binary ethosomes that are blank and then loading actively the drug. Phospholipid is then mixed at start in some alcoholic phase, that consists of the ethanol and Polyethylene glycol. Then, a citrate buffer solution is progressively further mixed in previous solution with some continuous mixing at the 700 rpm. This system is mainly kept about at 30 ± 1°C throughout the process and then this is cooled at the room temperature. This will complete the preparation of blank binary ethosomes. Then, drug is loaded in the ethosomes and system is progressively mixed at 700 rpm, so as to efficiently dissolve and disperse the active drug. pH gradient among internal phase (acid) and external phase (alkaline) of ethosomal formulation could be recognized by the addition of sodium hydroxide solution 0.5 M to manage the external pH. Later, the formulation was incubated for a suitable temperature and time, to let unionized drugs passes vigorously throughout the lipid bilayer of the ethosomes and then got ensnared in the vesicles (44, 88)

**CHARACTERIZATION OF ETHOSOMES**

**Zeta potential and vesicle size**

Zeta potential and size of the particles were determined, then examined by the dynamic light scattering, utilizing a electronic review system (89). Size of the vesicle is most sensitive parameter of the topical delivery system, e.g., the vesicles that are lesser than the 300 nm were delivering their contents, towards many degree, inwards the deeper films of the skin (90). Zeta potential and the size of the vesicles were determined by taking 1 ml of the nanoethosomal formulation and then was diluted with 10 ml of the double distilled water and is then analyzed by using the dynamic light scattering particle size analyzer, i.e., Malvern Zetasizer (10, 26).

**pH**

pH assumes a significant part in preparation just as upkeep of physical integrity of the vesicles just as the loading of the drug that is active into vesicles. Some fluctuations in the pH of that system can prompt and can variate pH of the polymer system, this influences the enlarging capacity. Ideal pH should be needed for stability of the vesicles (61, 91).

**Surface tension**

Ethanol and Surfactants decreases the surface tension within vesicles of formulation. The Interfacial tension could be assessed through estimating energy required for formation of the membrane. It is might impacted by the medium pH, proportion of the phospholipids, cholesterol, liquor, surfactants, additional drugs and some of the other cosolvents and so forth. This is a significant condition with respect to the fluidity as well as stability of vesicles. This has been reported that by reducing the interfacial tension, it is more suitable for better constancy of the oil-in-water emulsions. That is very vital for the stability of ethosomal formulation subsequently it consists of lipid in water. The surface tension as well as interfacial strain could be estimated with drop weight and drop volume method as well as with the capillary rise method (92). Recent studies had shown that the interfacial as well as the surface tension of the drug could be estimated and calculated by the use of ring method in Du Nouy ring tensiometer, drop weight, Wilhelm plate, drop volume and the capillary rise method (93).

**Transition temperature**

Transition temperature of vesicular system can be estimated by differential scanning calorimetry (DSC) under the continuous stream of nitrogen in the aluminium pan at about 10°C per min (34).

**Entrapment efficiency**

Entrapment capacity of the ethosomes could be estimated with the help of ultracentrifugation method (26). Entrapment efficiency of the vesicles is subject to the phospholipids utilized, vesicle size, proportion of the bilayer parts, ionic strength and pH of medium (94). This was estimated by isolating free medication of the vesicles utilizing some strategies like the dialysis, gel chromatography, ultracentrifugation, ultrafiltration, as well as small segment centrifugation. It is accounted for that examination of entrapment efficiency done by the ultracentrifugation is less explicit, delicate, and particular when contrasted with other different techniques (95). The sensitivity and poor selectivity are ascribed to deformity of vesicles during ultracentrifugation. Later partition, the vesicles were broken by utilizing N propanol or 0.1% Triton X-100. EE is determined with the help of formula provided in the equation (96-98)
EE = Quantity of the drug entrapped / Whole quantity of the drug loaded * 100  (Eq. 1)

**Drug content**
Recent studies have shown that the content of the drug within ethosomes could be examined with the utilizing of the UV spectrophotometer. That could be measured with the HPLC technique (89).

**Stability studies**
The Stability study of ethosomal preparation is the major of central point as this uncovers their capacity to hold the constitution alongside active pharmaceutical ingredient (API). The uncertainties in the ethosomal definitions were brought about with the oxidation or hydrolysis of phospholipid or these are evaluated with the outflow of uncoated medication and adjustments in the size because of aggregation and fusion. Modification in the size, size dispersion, entanglement productivity as well as total of the vesicles were vital conditions for observing the strength. All these of the parameters could be measured by transmission electron microscopy (TEM) or with the dynamic light dissipating (59, 99). Storage conditions of the ethosomal formulations can be examined with the comparison of size, shape and the entrapment size of vesicles after some suitable time on various storage conditions (31). The sample of niosomes at different intervals of time (0,1,2,and 3months) were also observed for the color change, characteristics of the surface and been tested for percent drug remain after being hydrated for the formulation of niosomes and is then analyzed with some suitable analytical methods such as the HPLC methods and UV spectroscopy, etc (100).

In view of different studies of stability performed, analysts propose refrigerated condition, i.e., 4-8°C, as one of the reasonable storage conditions of the ethosomal preparations. Increased temperatures might lead to degradation of the vesicular lipids, loss of the physical integrity of the vesicles and a quick leakage of the ensnared contents of the formulation (101). Drug loss from the vesicles stored at raised temperatures might be ascribed with the influence of the temperature on gel to the liquid transition of bilayers of lipid along with conceivable chemical decomposition of the phospholipids, prompting to flaws in the film packing (31).

**In-vitro permeation studies**
The In vitro permeation test could be accomplished using the membrane of the skin abdominal of the male rats with the phosphate buffer of about 7.4 pH as the recipient compartment. From this point onward, the skin of abdominal of rats is then sited in center of the recipient and the donor compartment with dermal side directly relates to the medium of the receptor (102, 103). 1 g of sample was then applied to the surface of the skin with the area of diffusion about 1.76 cm². Afterwards, the 4 ml samples were taken at the continuous intervals of about for 12 hours. The sample was diluted and was then measured by utilization of the UV-visible spectrophotometry (104). Afterwards, that concentration of drug in sample could be calculated, this could be measured by the cumulative quantity of drug penetrated utilizing below equation (105):

\[ Q_t = V_s C_i + V_r C_t \]  (Eq. 2)

Description: \( Q_t \) = Cumulative quantity of drug penetrated in \( \mu g \), \( V_r \) = Volume of the compartment (receptor) of Franz diffusion cell 16 mL, \( V_s \) = Volume of the sample 4 mL, \( C_t \) = Concentration at minute -n in ng/mL, \( C_i \) = The concentration of sample per minute.

By the above study obtained, cumulative quantity of the drug per unit area (\( \mu g/cm^2 \)) could also be calculated with below equation (106, 107),

\[ Q = \frac{Q_t}{S} \]  (Eq. 3)

Description: \( Q \) represents the Cumulative quantity per unit area (\( \mu g/cm^2 \)), \( S \) represents the Area of membrane (1.76 cm²), \( Q_t \) represents the Cumulative quantity penetrated (\( \mu g \)).

Moreover, by this analysis we got a graph of the drug concentration per unit area (ng/cm²) over time, we obtain single straight line, the slope of line represents rate of drug release (105).

**CONCLUSION AND FUTURE PERSPECTIVES**
Introduction of the novel ethosomes had now started another area of the vesicular studies for TDDS. Various results had shown some promising fate of the ethosomes in forming transdermal drug delivery of different drugs more compelling. More, exploration through this aspect will permit much better control of the release of drug in-vivo, permitting the doctor for making the treatment more viable. Ethosomes do offer a decent chance for the
non-invasive drug delivery of little, medium, and for the large size of drug molecules. Numerous different kinds of new vesicles that are elastic for example the penetration enhancement vesicles (PEV) and invasomes are continuously arising. Invasomes, the flexible vesicles where terpenes and ethanol are utilized, which upgrade skin pervasion, the stability and elasticity of vesicles. The permeation enhancers’ vesicles are those type vesicles wherein hydrophilic permeation enhancers like Labrasol, Transcutol and propylene glycol are included in the liposomes. The PEV vesicles had several advantages of different vesicles as these have combinatorial benefit of the liposome as transporter just as capacity of penetration enhancer to change layer corneum for the increased penetration. Further studies are to be done to have this vesicular system to be more effective and then could increase ultimately the patient compliance.

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MAW contributed to study concept, study design and data collection. MZ contributed in data analysis and interpretation. HH and MM did the literature review and critically reviewed the manuscript. All the authors read and approved the final manuscript.

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