Ethanol Based Vesicular Carriers in Transdermal Drug Delivery: Nanoethosomes and Transethosomes in Focus

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Abstract

Transdermal route is one of the attractive routes for drug delivery due to its easy accessibility. Effective delivery of bioactive molecules through skin is however, still a challenge. The development of vesicular formulations has generated some promising solutions to the problems associated with drug delivery not only related to drugs but also those of barriers like skin. Conventional lipid based vesicular systems like liposomes show inability to cross intercellular channels of stratum corneum. To overcome this drawback of conventional lipidic systems, ethanol based vesicular carriers were developed by pharmaceutical scientists. Nanoethosomes and transethosomes come under the category of ethanol based lipidic carriers. Nanoethosomes are composed of phospholipid, ethanol and water, while transethosomes have exactly same composition but additionally they contain edge activators (like span 60) or permeation enhancers (like oleic acid). Ethanol based vesicular systems represent non-invasive carriers which enable the drug to reach in deeper epidermal layers or systemic circulation. The nature, methods of preparation, and evaluation parameters of nanoethosomes and transethosomes were discussed in this review along with their applications, problematic issues and future progress.

Keywords

Edge activator, Nanoethosomes, Transethosomes, Transdermal, Vesicular

Introduction

In current scenario transdermal delivery of bioactive molecules has become an interesting research area; however, effective transdermal drug delivery is still a challenge. Various approaches explored for transdermal delivery which overcome barrier functions of skin include electrically assisted methods (sonophoresis, iontophoresis, and electrophoresis), micro-invasive techniques, vesicular systems, and use of chemical permeation enhancers [1]. Transdermal delivery enables direct entry of bioactive molecules into systemic circulation, bypass of hepatic metabolism [2], improvement of patient compliance, and low risk of injury to the tissues [3]. A bioactive molecule should have characteristics like low molecular weight (<500 Da), high pharmacological activity, high effectiveness at low doses (5-10 mg/day), and high lipophilicity for achievement of good results following transdermal administration [4]. Various classes of drugs fulfilling these criterions are analgesics, contraceptives, antianginals, and antihypertensive drugs [5]. Vesicular system is most widely investigated approach for transdermal drug...
Various formulation ingredients of nanoethosomes and their role

Nanoethosomes have phospholipids, ethanol, and water as main formulation ingredients. Phospholipids have an integral role in bilayer formation; consisting of hydrophilic head and hydrophobic tail. Commonly used phospholipids in nanoethosomes manufacturing are phosphatidylycholine (PC), soybean phosphatidylycholine (Phospholipon 90), and phosphatidylethanolamine (PE) [19]. Alcohol is a central character of nanoethosomal system giving unique identity to it as a vesicular system. Impact of ethanol on different lipidic system was studied during the last decade of nineteenth century [20]. Lipidic layer of stratum corneum are fluidized by impact of ethanol and its high concentration in nanoethosomes promote malleability and flexibility of these systems promoting their penetration through tiny openings formed in stratum corneum due to fluidization [21]. Alcohol amount in vesicular system also control its diameter as it provides net negative charge to vesicle surface reducing its size [22]. 30-40% is optimum concentration range of ethanol for the formation of stable ethosomes [23]. Reducing ethanol concentration to 20% may leads to increase in vesicular size [14]. Sometimes, skin permeation enhancers like transcutol and polyols (propylene glycol) are also used for nanoethosome formulation. Permanence of ethosomal membrane is maintained by adding small amount of cholesterol [24].

Part A: Nanoethosomes as Transdermal Drug Carriers

Nanoethosomes are nanosized lipid based vesicular carriers having high concentration of ethanol used for deeper skin permeation of bioactive molecules [10]. The main components of nanoethosomes are phospholipids, ethanol, and water. Presence of high amount of ethanol in their structure differentiates them from other vesicular systems and also helps to release encapsulated material into basal skin layer and blood circulation [11]. First time development of ethosomes was carried out by Touitou in 1996 for skin permeation enhancement [12]. Figure 1 gives structural elucidation of nanoethosomes. Nanoethosomes are soft and malleable in nature. Size of nanoethosomes lies in nanometer range; although, it is dependent on phospholipid concentration used [10]. High alcohol content in nanoethosomes may be another factor for their reduced size compared to liposomes prepared under same conditions. Ethanol gives a net negative charge on vesicle surface promoting its size reduction [13]. Nanoethosomes penetrate through intercellular pathway in the stratum corneum (Figure 2) [4].

Advantages of nanoethosomes as transdermal drug carrier

Pharmaceutically adequate excipients are used in formulation of nanoethosomes. Their scaling up is simple and less elaborate procedures are involved in their manufacturing [14]. Nanoethosomes are biodegradable in nature and high alcohol content gives a negative charge to them restricting their vesicular size low; leading to high penetration and enhanced bioavailability of bioactive molecules [15]. Nanoethosomes show high encapsulation efficiency for wide variety of molecules including lipophilic drugs [16]. Drug loaded nanoethosomes can be easily dispersed in cream or gel; therefore, providing high patient compliance compared to electrically assisted techniques like iontophoresis [17]. Ethosomes involve less toxicity concerns due to well acknowledged toxicity profiling of formulation ingredients in the scientific literature [18].

Figure 1: Schematic structure of nanoethosomal system. Water and ethanol with drug molecule reside as a core in phospholipid bilayer. Some amount of ethanol can occupy bilayered region also.

Figure 2: Intercellular permeation of nanoethosomes in skin. Fluidization caused by ethanol may increase intercellular space between corneocytes.
Preparation techniques of nanoethosomes

Nanoethosomes are prepared by using cold technique and hot technique.

Cold technique: This technique is most widely used of preparation of nanoethosomes. This method involves dissolution of lipidic materials in ethanol with continuous stirring at room temperature followed by the addition polyol solution and heating up to 30 °C with vigorous agitation [21]. Mixture is stirred for 5 minutes in a covered vessel. Furthermore, sonication is done to decrease the size of nanoethosomes [25].

Hot technique: In this technique phospholipid is dispersed in water and heated up to 40 °C for the formation of colloidal dispersion. Furthermore, mixture of polyol and ethanol are heated up to 40 °C in a separate container. Both solutions are then mixed with each other by continuous stirring. Depending upon hydrophilic or lipophilic nature of drug; it is either dissolved in water or ethanol. Probe sonication of mixture is carried out later on to get nanoethosomes of desired size [4]. Figure 3 describes preparation of nanoethosomes by hot and cold techniques.

Characterization parameters of nanoethosomes

Morphology of nanoethosomes: Morphology of nanoethosomes can be studied by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) [26]. TEM involves drying of samples on carbon coated grid and negative staining with aqueous solution of phosphotungstic acid. Furthermore, samples are dried and observed under high magnification at an accelerating voltage of 100 kV. SEM involves mounting of ethosomal solution on clear glass stub, air drying, and coating with Polaron E 5100 Sputter coater, and visualization under microscope [27].

Nanoethosomal size and size distribution: Vesicle size and size distribution of nanoethosomes can be determined by using dynamic light scattering (DLS) technique [28]. For DLS investigations; mixing of nanoethosomal suspension is carried out with appropriate medium [usually phosphate buffer saline (PBS)] [27].

Encapsulation efficiency: Encapsulation efficiency of nanoethosomes can be determined by using ultracentrifugation or dialysis bag method.

Ultracentrifugation: In this method, prepared nanoethosomal formulation is kept overnight and then subjected to ultracentrifugation at specific RPM for calculated period of time. Samples are assayed using high-performance liquid chromatography (HPLC) [4]. Following formula is used to calculate encapsulation efficiency (EE):

\[
\text{Encapsulation efficiency} = \frac{\text{Theoretical amount of drug added}}{-\text{Amount of drug detected}} \times 100
\]

Dialysis bag method: In this method dialysis bag made up of cellulose acetate are used for the study. Bags are kept in saline solution for 1 h prior to use for wetting of membrane. A specific amount of drug loaded vesicles are then placed into dialysis bag following its transfer to phosphate buffer saline (500 mL) of a specific pH. Receiver medium is subjected to continuous magnetic stirring. Samples withdrawn from receiver at regular time interval are analysed by using HPLC. EE is calculated by using formula given above [13]. Maheshwari et al. [29] prepared ethosomes and ultraformable liposomes of clotrimazole for transdermal drug delivery. They calculated entrapment efficiency of prepared ethosomes by ultracentrifugation method and found that at vesicle size of 132 ± 9.5 nm; ethosomes showed 68.7 ± 1.4% entrapment of drug.

Calorimetric analysis: Calorimetric analysis of nanoethosomes is carried out to determine the transition temperature (Tm) of vesicular lipids in them. Low Tm value indicates fluidizing effect of ethanol on phospholipid bilayer [27]. Differential scanning calorimetry (DSC) is carried out with a programmed heating rate of 10 °C per minute under a constant stream of nitrogen in range of –50 °C to 50 °C [30].

Permeation studies of nanoethosomes (Drug release studies)

Ethanol is a well-established permeation enhancer. High permeation of nanoethosomes in skin may be due to synergistic effect of ethanol and vesicular lipids. Human cadaver skin from abdominal areas, rat skin, or guinea pig
skin may be a choice to carry out permeation studies. After selecting, skin is mounted on the Franz diffusion cell along with subcutaneous side facing towards donor compartment. Near about 5 mL of PBS (pH 5.4) is localised in receptor compartment and subjected to magnetic stirring at 100 RPM. 100 µL nanoethosomal formulations is applied to donor compartment of Franz diffusion cell maintained at 32 °C ± 1 °C. Samples withdrawn at specific time intervals are analysed using HPLC [29]. Verma and Pathak [31] prepared nanoethosomes loaded with econazole nitrate and studied their skin penetration in gel form using Franz diffusion cell in rat skin. It was reported that ethosomal formulation could penetrate upto stratum basale layer of epidermis.

Measurement of depth of skin penetration of nanoethosomes

In order to measure depth of skin penetration of nanoethosomes, confocal laser scanning microscopy (CLSM) technique is used [31]. A fluorescent probe like Rhodamine 123 or Rhodamine-red are generally employed to detect penetration depth by loading them in nanoethosomes [32]. Probe loaded formulation is applied to skin sample maintained at 37 °C. Excess of formulation is removed from skin sample and it is scanned at different increments along with Z-axis of the CLSM microscope [27]. Chourasia et al. [33] performed CLSM study of nanoethosomes prepared for transdermal delivery of ketoprofen using Rhodamine 123 as a fluorescent probe. Result of study predicted that nanoethosomes could penetrate upto 40 µm in skin.

Stability studies of nanoethosomes

Stability study of nanoethosomes is performed by monitoring size, morphology, and drug leakage after its storage at a specific temperature for specified time period. For the purpose, nanoethosomes are kept in sealed vials of 10 ml capacity after flushing with nitrogen [34]. Dubey et al. [35] performed stability studies of methotrexate (MTX) loaded nanoethosomes. They evaluated stability profile of nanoethosomal formulations at different temperatures and found lowest drug leakage at refrigerated condition (RF).

Applications of nanoethosomes in transdermal drug delivery

Nanoethosomes can enhance drug delivery efficiency more than 65% due to their distinct capability to bypass the intact human skin [10]. So, nanoethosomes have been investigated for transdermal delivery of various bioactive molecules.

Delivery of antifungal drugs: Bhalaria et al. [36] prepared fluconazole loaded nanoethosomes and evaluated their clinical efficacy in patients with cutaneous candidiasis. At the optimized size (144 ± 6.8 nm) and entrapment (82.68%); ethosomes showed high clinical efficacy compared to liposomal formulation, marketed formulation and hydroethanolic solution of the drug. Furthermore, the transdermal efficacy of ciclopirox olamine loaded ethosomes was evaluated by Girhepunje et al. [37]. Formulation having 45% ethanol content showed highest entrapment (72.81 ± 3.5%) and optimized size (152 ± 11 nm). Results of CLSM study revealed permeation of ethosomes upto 168 µm in the rat skin. Table 1 gives brief information about research investigations performed over nanoethosomes for delivery of antifungal drugs.

Delivery of anti-inflammatory drugs: Paolino et al. [30] prepared ammonium glycyrrhizinate loaded ethosomes and

Table 1: Applications of nanoethosomes for transdermal delivery of antifungal drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Excipients</th>
<th>Sophisticated Techniques used</th>
<th>Entrapment/size/PDI (polydispersity index)/animal model</th>
<th>Key findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td>Soy phosphatidyl choline, propylene glycol, ethanol</td>
<td>Transmission electron microscopy (TEM), Confocal laser scanning microscopy (CLSM)</td>
<td>71.56% ± 218.4 ± 2.9 nm/ 0.451 ± 0.03/------</td>
<td>Drug loaded nanoethosomes showed high drug entrapment, greater penetration power, and high stability compared to liposomes</td>
<td>[38]</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>Soybean phosphatidyl choline (Phospholipon 90 (H), ethanol)</td>
<td>TEM, Atomic force microscopy (AFM), FT-IR spectroscopy</td>
<td>68.73 ± 1.4%/ 132 ± 9.5 nm/ 0.027 ± 0.011/ Sprague Dawley rats</td>
<td>Nanoethosomes showed high drug entrapment, enhanced transdermal permeation flux, and in-vitro antifungal activity compared to ultra deformable liposomes; along with high zone of inhibition compared to marketed formulation</td>
<td>[29]</td>
</tr>
<tr>
<td>Econazole nitrate (EN)</td>
<td></td>
<td>TEM, High performance liquid chromatography (HPLC), CLSM</td>
<td>81.05 ± 0.13%/ 202.85 ± 5.10 nm/ 0.37 ± 0.01/ Wistar albino rats</td>
<td>Optimized nanoethosomal gel showed controlled release for 12 h, two folds higher diffusion across rat skin, and high stability compared to liposomal and hydroethanolic gels</td>
<td>[31]</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>Cavamax (W6, W7, and W8), propylene glycol, Ethanol, triethanolamine, iso-propyl myristate</td>
<td>TEM, CLSM</td>
<td>98.42 ± 0.15%/ 202.8 ± 4.8 nm/ 0.113 ± 0.02/ Wistar albino rats</td>
<td>Cavnax W7 composite ethosomal gel showed high drug permeation flux, deeper penetration in epidermis, and high antifungal activity against Candida albicans and Aspergillus niger compared to normal ethosomal gel</td>
<td>[24]</td>
</tr>
<tr>
<td>Griseofulvin</td>
<td>Phospholipon 90G, Carbopol 980 NF, ethanol</td>
<td>TEM, Fluorescence microscopy, Reverse phase HPLC</td>
<td>72.94 ± 0.80%/ 148.5 ± 0.48 nm/ 0.20/ Laca mice</td>
<td>Griseofulvin-loaded ethosomes completely cured fungal infection in guinea pigs in 8 days upon twice daily topical applications</td>
<td>[39]</td>
</tr>
</tbody>
</table>
investigated anti-inflammatory activity in human volunteers. Ethosomal suspension with high ethanol content (45% v/v) and low lecithin content (2% w/v) showed high in-vitro percutaneous permeation, good skin tolerability, and in-vivo anti-inflammatory activity in humans. Later on, Zhouwu et al. [44] prepared matrine loaded nanoethosomes and investigated their percutaneous permeation capacity in-vitro and anti-inflammatory activity in-vivo. Nanoethosomes showed decrease in size with an increase in ethanol content; while an entrapment efficiency, increase within the increase in concentration of ethanol and phospholipid both. Matrine loaded nanoethosomes more effectively reduced induced erythema and inflammation in rat skin compared to naneothosomal formulations. Role of nanoethosomes in effective transdermal delivery of other anti-inflammatory drugs is explained in Table 2.

Table 2: Role of nanoethosomes for transdermal delivery of anti-inflammatory drugs.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Excipients</th>
<th>Sophisticated Techniques used</th>
<th>Entrapment/size/ PDI (polydispersity index) /animal model</th>
<th>Key findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aceclofenac</td>
<td>Soybean phosphatidylcholine, isopropyl alcohol</td>
<td>Scanning electron microscopy (SEM)</td>
<td>95.7%/ 0.696 µm /-- /-- /------</td>
<td>Nanoethosomal formulation showed very high transdermal flux and high stability for 45 days compared to an ethanolic drug solution</td>
<td>[41]</td>
</tr>
<tr>
<td>Triptolide</td>
<td>Dipalmitoyl phosphatidyl choline, cholesterol, ethanol</td>
<td>HPLC</td>
<td>98.8 ± 4.7%/ 123.1 ± 8.6 nm/ 0.335/ Sprague Dawley rats</td>
<td>Nanoethosomal formulation showed highest in-vitro accumulation of Triptolide in skin and significant reduction in erythema in-vivo in rat model</td>
<td>[42]</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Soya phosphatidyl choline, cholesterol, ethanol</td>
<td>TEM, CLSM, HPLC</td>
<td>78.7 ± 4.9%/ 120.3 ± 6.1 nm/------/Adult Chinese female skin</td>
<td>Nanoethosomal formulation showed high transdermal flux and high in-vitro penetration compared to hydroethanolic solution of drug through human skin</td>
<td>[33]</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>45% phosphatidylcholine and 10–18% phosphatidyethanolamin, Diethylene glycol, cholesterol, ethanol</td>
<td>TEM,X-ray diffraction (small and wide angle X-ray scattering SAXS and WAXS), HPLC</td>
<td>55 ± 2.5%/ 95 ± 1.8 nm/ 0.20/ Female CD-1 mice</td>
<td>Results of in vivo and ex vivo showed capability of all vesicular systems especially PEVs (penetration enhancer-containing vesicles) to localize drug at inflammation site compared to marketed formulation (Voltaren) in mice skin</td>
<td>[43]</td>
</tr>
<tr>
<td>Diclofen sodium</td>
<td>Soya lecithin, cholesterol, ethanol</td>
<td>Photon correlation spectroscopy</td>
<td>51.72 ± 4.36%/ 202 ± 20.6 nm/ 0.34/ Sprague Dawley rats</td>
<td>Nanoethosomal formulation showed high permeation through rat skin and permeability coefficient of nanoethosomes was 15 folds higher than conventional liposomes</td>
<td>[44]</td>
</tr>
</tbody>
</table>

Delivery of antiviral drugs: Jain et al. [25] developed lamivudine loaded nanoethosomes for effective transdermal delivery and evaluated them for cellular uptake study. Prepared ethanolic formulation showed twenty-five times more transdermal flux in rat skin compared to plain drug solution. Inter cellular uptake of ethosomes was five times more in T-lymphoid cell line (MT-2) compared to free drug solution. Later on, production and in-vitro activity evaluation of anti-HSV-1 molecules [acyclovir (ACY) and N1-beta-D-ribofuranosyl-pyrazole [3, 4d] pyridazin-7(6p-chlorine-phenyl)-one nucleoside (N1CP)] loaded nanoethosomes was carried out by Cortesi et al. [47]. Nanoethosomes showed controlled release of both molecules predicted through Franz diffusion cell study. Plaque reduction assay in monolayer cultures of Vero cells showed reduction in the ED50 of N1CP indicating increase of its antiviral activity. However, ACY remained more active than N1CP. Investigations done on other antiviral drugs delivered through nanoethosomes are shown in Table 4.
Delivery of other bioactive molecules/drugs: Dayan & Touitou [51] prepared trihexyphenidyl HCl (THP) loaded nanoethosomes and evaluated them for transdermal penetration in mice skin using CLSM technique. Nanoethosomes of drug showed 87 and 4.5 times higher transdermal flux compared to conventional liposomes and hydroethanolic solution respectively, and nanoethosomes also showed high depth of penetration compared to conventional liposomes. Bacitracin loaded nanoethosomes were evaluated by Touitou et al. [1] for intracellular delivery following transdermal route. Results of fluorescent-activated cell sorting (FACS) study showed effective penetration of nanoethosomes through cellular membrane with the release of entrapped bacitracin within the cells. Later on, Dubey et al. [52] evaluated transdermal potential of melatonin (MT) loaded nanoethosomes in human cadaver skin and compared them with conventional liposomes. Results of FT-IR studies revealed high mobility of skin lipids after application of nanoethosomes compared to liposomes; and, nanoethosomes also showed penetration upto 240 µm in human cadaver skin. Table 5 gives brief information about research investigations performed over nanoethosomes for delivery of various types of drugs.

Intellectual property rights (IPRs) related to nanoethosomes for transdermal drug delivery

Descriptions of various patents related to nanoethosomes for transdermal drug delivery are given in Table 6 [63]. Nanoethosomes were firstly patented by Prof. Elka Touitou of Hebrew University School of Pharmacy, Jerusalem in 1996 [12]. After publication of this patent, several patents related to nanoethosomes were filed and granted. Patents related to nanoethosomes have opened a new window for the entry of new technologies for drug carriers in pharmaceutical market. Various marketed nanoethosomal products like Nanominox™ (minoxidil containing nanoethosomes, produced by Sinere, Germany) and Noicellex™ (topical anti-cellulite cream, produced by Novel Therapeutic Technologies, Israel) are results of patents granted on nanoethosomes [4].

Part B: Transethosomes as Transdermal Drug Carriers

Liposomal carriers have been studied for transdermal drug delivery since the 1980s. Conventional liposomes (CLs) show drawback of less permeation into the deeper region of skin and they accumulate at the outer layer of stratum corneum...
**Table 5: Applications of nanoethosomes for transdermal delivery of various bioactive molecules.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Excipients</th>
<th>Sophisticated Techniques used</th>
<th>Entrapment/size/ PDI (polydispersity index)/animal model</th>
<th>Key findings</th>
<th>Reference</th>
</tr>
</thead>
</table>
| 5-Aminolevulinic Acid (ALA) | Phosphatidyl ethanolamine, ethanol | Colorimetry, CLSM, HPLC                                             | 67.51%/ 156.3 nm/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/-/
Table 6: Various patents related to nanoethosomes for transdermal drug delivery.

<table>
<thead>
<tr>
<th>Title of patent</th>
<th>Brief description</th>
<th>Inventors</th>
<th>Patent Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethosome preparation of male hormone medicaments and its preparation method</td>
<td>This invention describes the preparation technique of ethosomes with male hormone used to treat various male diseases like male sterility, endocrine erectile dysfunction, and male climacteric syndrome</td>
<td>Guan Yan Min, Meng Shu, Li Jianxin, Dan</td>
<td>CN102406605 A</td>
</tr>
<tr>
<td>Progesterone ethosome, and Transethosomes in Focus</td>
<td>This invention describes a method of encapsulation of progesterone (0.1%-1%) in ethosomes for treatment of secondary amenorrhea, dysfunctional bleeding, and premenstrual syndrome</td>
<td>Zhang Shu, Deng Hong, Lin Huaqing, Zhang Xiaoling</td>
<td>CN102397255 B</td>
</tr>
<tr>
<td>Transdermal composition for treating pain</td>
<td>This invention describes ethosomal composition for transdermal delivery for treatment of pain; the present invention can be used to treat different type of pain like muscular, nociceptive, and neuropathic in origin</td>
<td>Moheb Maalawy</td>
<td>WO2015123750 A1</td>
</tr>
<tr>
<td>Preparation method of lidocaine ethosome</td>
<td>This invention discloses a method of preparation of lidocaine ethosomes using lecithin and ethanol as major constituent; prepared ethosomes showed entrapment up to 80.93% and good skin compatibility</td>
<td>Liang Ju, Wu Wenlan, Li, Miao Juan, Wei Xuefeng, Chen Shan, Wang Xiaotao</td>
<td>CN102688194 B</td>
</tr>
<tr>
<td>Daptomycin ethosome preparation</td>
<td>This invention describes preparation method of daptomycin ethosomes using 1 mg daptomycin, 10–20 mg lecithin, 0.6–0.8 ml ethanol, and balance of water; the ethosomes show low preparation cost and high stability</td>
<td>Lee Chong, Liu Ha, Yanqi Kun, Wang Xiaoying, Chen Po</td>
<td>CN103006562 B</td>
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<tr>
<td>Phenasteroid gel preparation</td>
<td>This invention discloses preparation method of phenasteroid using 0.5–4% phospholipid and their dispersion in carborner (0.25–1.5%) gel for topical application</td>
<td>Liang Wen- right, Rao Yuefeng</td>
<td>CN1555804 A</td>
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<tr>
<td>Bullatacin ethosome gel and preparation method thereof</td>
<td>This invention describes method of preparation of ethosomal gel using Brad he octyl, phospholipid, low molecular weight alcohol, cholesterol, stabilizer, and antioxidant; size of ethosome is 30–400 nm</td>
<td>Tan Jiaping, Jiang Lixin, often calm, Zhou Zhiven</td>
<td>CN102552147 B</td>
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<tr>
<td>Acyclovir ethosome and preparation method thereof</td>
<td>This invention discloses acyclovir loaded ethosomes with improved stability by addition of polyethylene glycol or chitosan for percutaneous administration</td>
<td>Wuxue Wen, Xiong Yan</td>
<td>CN102133183 B</td>
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<tr>
<td>Ethosomes preparation of antymycotics pharmaceutical and method for preparing the same</td>
<td>This invention discloses an ethosomal preparation loaded with antifungal drug containing 1 to 8% phospholipid, 20 to 45% ethanol, and 40.9 to 78.9% of water</td>
<td>Liu Liping, Li Yimin, Shen Ming- high, six Jiang Hu, Yang Jia</td>
<td>CN101273971 A</td>
</tr>
<tr>
<td>Clotrimazole ethosomes for preventing and curing weaning rabbit dermatomycosis and preparation method thereof</td>
<td>This invention describes the composition and method of preparation of ethosomes loaded with clotrimazole having 3% of lecithin and 1% of clotrimazole by weight</td>
<td>Li Man, Mou special, Liming Yong</td>
<td>CN104873465 A</td>
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</tbody>
</table>

Table 7: Brief overview of research work done on transethosomes for transdermal drug delivery.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Excipients</th>
<th>Sophisticated Techniques used</th>
<th>Entrapment/size/PDI (polydispersity index/animal model)</th>
<th>Key findings</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voriconazole</td>
<td>Lipoid S100, Cholesterol, Tween 80, Taurocholic acid sodium, Ethanol</td>
<td>TEM, HPLC</td>
<td>96.6 ± 2.7%/ 191.9 ± 41.5 nm/ -----/Male albino mice</td>
<td>Prepared transethosomes showed high elasticity, high in-vitro skin permeation, and high in-vivo skin deposition of voriconazole compared to nanoethosomes and conventional liposomes</td>
<td>[68]</td>
</tr>
<tr>
<td>Ketorolac Tromethamine</td>
<td>Phospholipon 90G, Sodium deoxycholate, Propylene Glycol, Ethanol</td>
<td>TEM, FT-IR</td>
<td>82.08 ± 4.5%/ 180 ± 70 nm/ -----/Male albino rats</td>
<td>Transethosomes showed 3 fold more elasticity compared to ethosomes and transethosomal gel 3 fold increase in transdermal flux compared to conventional ethosomes</td>
<td>[69]</td>
</tr>
<tr>
<td>Vitamin E/caffeine</td>
<td>Soybean phosphatidyl choline, Sodium cholate, ethanol</td>
<td>TEM, HPLC</td>
<td>For vitamin E - 76.68% ± 2.94%/ 154.73 ± 1.89 nm/ 0.428 ± 0.020/Pig ear skin For caffeine - 3.376 ± 0.812%/ 116.60 ± 2.25 nm/0.133 ± 0.015/Pig ear skin</td>
<td>For transdermal flux and stability, order obtained was: transethosomes (TE) &gt; ethosomes (E) ≥ transfersomes (T) for both vitamin E and caffeine</td>
<td>[72]</td>
</tr>
</tbody>
</table>
like oleic acid [68]. Figure 4 shows formulation ingredients of transeosones.

**Method of preparation of transeosones (TELs)**

For the preparation of TELs, phospholipid, edge activator (or permeation enhancer), and drug (if lipophilic) is dissolved in ethanol. Further, double distilled water (DDW) (containing drug if it is hydrophilic) is added to an ethanolic solution under mixing at near about 700 RPM with a magnetic stirrer followed by homogenization at 10000 RPM for 1 minute. The resulting mixture is then continuously mixed for 10 min and filtered through a membrane filter. Prepared TELs formulation is stored at room temperature [69]. Characterization parameters of TELs are same as that of ethosones.

**Research investigations proving transeosones better compared to nanoethosomes**

Garg et al. [70] prepared transeosones loaded with piroxicam and compared them in gel form with liposomes, ethosones, and transferosomes. Optimized transeosones showed highest entrapment, elasticity, and improved stability compared to all other vesicular systems. Transeosomadal gel showed highest drug permeation compared to other gel formulations. A comparative assessment between imiquimod loaded transeosones and nanoethosomes for transdermal delivery was carried out by Ma et al. [71]. Transeosones showed high accumulated drug (24.64 µg/cm²) and local accumulation efficiency (6.70) compared to conventional ethosones (14.45 µg/cm² and 3.93, respectively). Results of CLSM study revealed deeper skin penetration of transeosones compared to conventional ethosones [71]. Table 7 gives brief overview of research work done on transeosones for transdermal drug delivery. No intellectual property rights (IPR) was found regarding transeosones in our literature survey.

**Problematic issues and future progress related to ethanol based carrier systems for transdermal drug delivery**

Most of the bioactive molecules do not pass through stratum corneum barrier. Ethanol based nanocarriers have opened a new window to deliver various bioactive molecules transdermally as they have capability to fluidize and disturb the rigid lipid system of stratum corneum. These systems represent an efficient non-invasive drug delivery approach for medium and large sized bioactive molecules along with high patient compliance and low cost treatment. However, effective clinical exploration of the ethanol based nanocarrier system is still a challenge. It is necessary to evaluate them clinically to check their potency. Ethanol based nanocarriers need safety exploration in some specific clinical conditions like their application to open areas of eczema as ethanol show irritant effect to skin. So, further research in this field will promote effective drug release in-vivo and make transdermal therapy more effective.

**Conclusions**

The development of ethanol based vesicular carriers like nanoethosomes and transeosones is a promising approach for delivery of large, small, soluble as well as insoluble bioactive molecules. Ethanol based carriers have capability to mask both drug related and physiological problems like first pass effect, short half-life, GIT irritation, less penetration, etc. Nanoethosomes have shown high transdermal flux of various bioactive molecules compared to conventional liposomes or hydro alcoholic solution. Transeosones are even better than nanoethosomes if used for the same purpose as proved in literature survey. Improvement in stability is a parameter of consideration for ethanol based carriers as they degrade due to oxidation of lipid/ phospholipid content. For their optimum stability necessary storage condition is at 4-8 °C. Formulation of gel of ethanolic vesicular carriers may improve their viscosity and hence increase their residence time at the application site like skin. So, ethanolic vesicular carriers have potential applications in the field of nanomedicine to deliver drugs having solubility/permeability problems through transdermal route.

**Disclosure Statement**

The authors confirm that this article content has no conflicts of interest.

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23. Bendas ER, Tadros MI. 2007. Enhanced transdermal delivery of


Ethanol Based Vesicular Carriers in Transdermal Drug Delivery: Nanoethosomes 
and Transethosomes in Focus

Kumar et al.


63. Patents related to nanoethosomes for transdermal drug delivery.


