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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

delivery nowadays. Vesicles are colloidal systems in which hydrophilic core is surrounded by amphiphilic molecules in a double layered fashion [6]. Vesicular formulations have become centre of attraction for pharmaceutical scientists to be used as effective transdermal drug carriers. Vesicular systems have capability to encapsulate wide variety of drug viz. hydrophilic, lipophilic, charged hydrophilic, and amphiphilic as well [7]. Effectiveness of a vesicular system as a carrier depends on various physicochemical characteristics like surface charge, size, elasticity, thermodynamic phase, and lamellarity. Chemical composition of vesicular systems governs these characteristics [8]. Several research reports of past a few years confirm the effectiveness of vesicular systems as transdermal drug carriers [9]. The present review gives a detailed knowledge of ethanol based vesicular carrier systems like nanoethosomes and transethosomes for efficient transdermal passage of various drugs.

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 elaborative 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].

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 phosphatidylcholine (PC), soybean phosphatidylcholine (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 ninetieth 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].

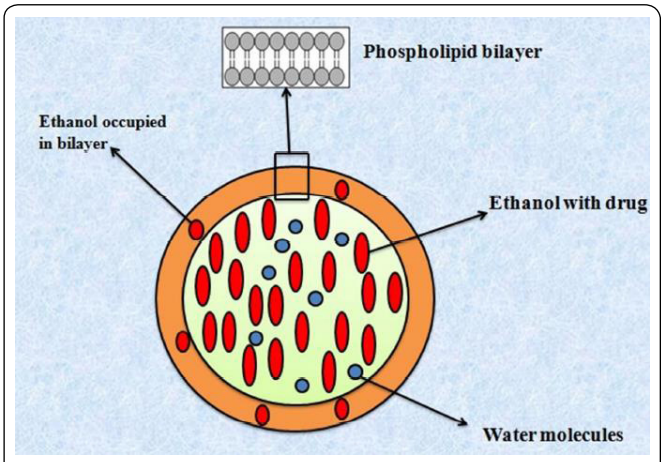

Figure 1: Schematic structure of nanoethosomal system. Water and ethanol with drug molecule reside as a core in phospholipid bilayer. Some amount of ethanol can occupy bilayered region also.

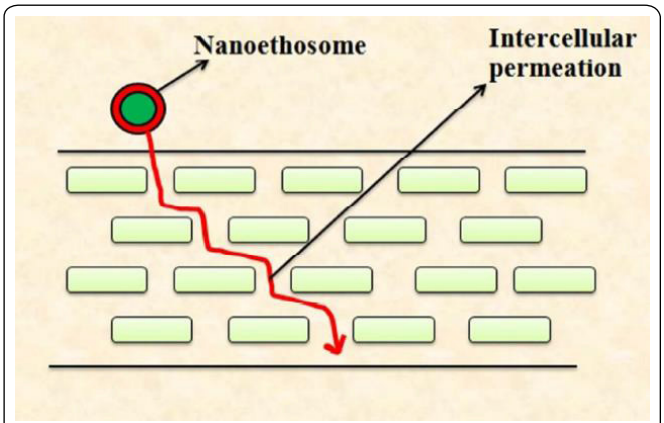

Figure 2: Intercellular permeation of nanoethosomes in skin. Fluidization caused by ethanol may increase intercellular space between corneocytes.

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 dissolvation 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 upto 40 °C for the formation of colloidal dispersion. Furthermore, mixture of polyol and ethanol are heated upto 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.

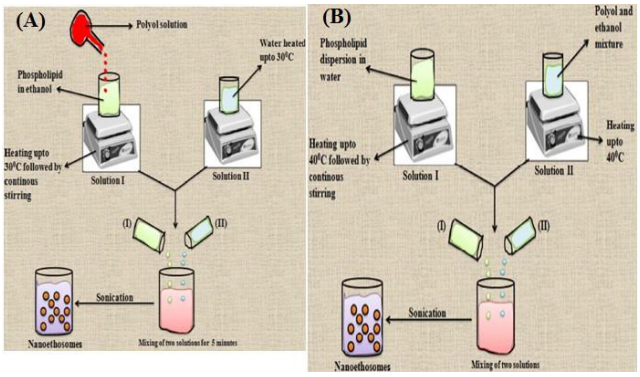

Figure 3: Preparation of nanoethosomes by cold and hot technique. (A) Cold technique involves mixing of two solutions at a low temperature of 30 °C, and (B) hot technique involves mixing of two solutions at a high temperature of 40 °C.

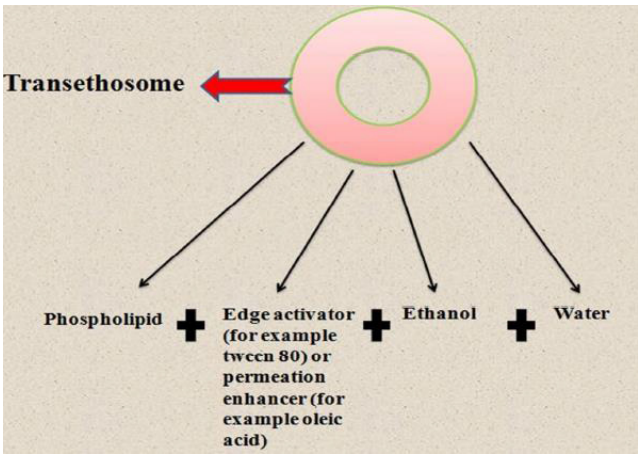

Figure 4: Composition of transethosomes. Transethosomes have high ethanol content along with either presence of edge activator or permeation enhancer.

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):

$$\text{Encapsulation efficiency} = \frac{\text{Theoretical amount of drug added-Amount of drug detected in supernatant}}{\text{Theoretical amount of drug added}} \times 100$$

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 ultradeformable 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 cilcopirox 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.

Drug	Excipients	Sophisticated Techniques used	Entrapment/size/PDI (polydispersity index)/ animal model	Key findings	Reference
Amphotericin B	Soya phosphatidyl choline, propylene glycol, ethanol	Transmission electron microscopy (TEM), Confocal laser scanning microscopy (CLSM)	71.56%/ 218.4 ± 2.9 nm/ 0.451 ± 0.03/------	Drug loaded nanoethosomes showed high drug entrapment, greater penetration power, and high stability compared to liposomes	[38]
Clotrimazole	Soybean phosphatidyl choline (Phospholipon 90 (H), ethanol	TEM, Atomic force microscopy (AFM), FT-IR spectroscopy	68.73 ± 1.4%/ 132 ± 9.5 nm/ 0.027 ± 0.011/ Sprague Dawley rats	Nanoethosomes showed high drug entrapment, enhanced transdermal permeation flux, and *in-vitro* antifungal activity compared to ultradeformable liposomes; along with high zone of inhibition compared to marketed formulation	[29]
Econazole nitrate (EN)		TEM, High performance liquid chromatography (HPLC), CLSM	81.05 ± 0.13%/ 202.85 ± 5.10 nm/ 0.37 ± 0.01/ Wistar albino rats	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	[31]
Clotrimazole	Cavamax (W6, W7, and W8), propylene glycol, Ethanol, triethanolamine, iso-propyl myristate	TEM, CLSM	98.42 ± 0.15 %/ 202.8 ± 4.8 nm/ 0.113 ± 0.024/ Wistar albino rats	Cavamax 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	[24]
Griseofulvin	Phospholipon 90G, Carbopol 980 NF, ethanol	TEM, Fluorescence microscopy, Reverse-phase HPLC	72.94 ± 0.80%/ 148.5 ± 0.48 nm/ 0.203/ Laca mice	Griseofulvin-loaded ethosomes completely cured fungal infection in guinea pigs in 8 days upon twice daily topical applications	[39]

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, Zhaowu et al. [40] 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 nanoethosomal 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.

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

Delivery of cardiovascular drugs: Touitou et al. [10] investigated minoxidil loaded nanoethosomes for transdermal delivery. Prepared nanoethosomal formulation at 2% phosphatidylcholine and 30% ethanol showed rapid enhancement in transdermal permeability of compared hydroethanolic or phospholipid ethanolic solution of minoxidil. Furthermore, Ahad et al. [45] investigated skin penetration capacity of valsartan loaded nanoethosomes using CLSM and pharmacokinetic behavior in Wistar albino rats. Results of study showed penetration of nanoethosomes in deeper skin layers compared to conventional liposomes and 3.03 times increase in bioavailability compared to oral suspension of valsartan. Later on, preclinical evaluation of valsartan loaded nanoethosomes was carried out by Bhosale and Avachat [46] in wistar albino rats. Nanoethosomes showed a prolonged antihypertensive effect in wistar rats following transdermal application compared to orally administered drug suspension. Histopathological investigation showed dissolvation of intercellular lipids of epidermis by nanoethosomes promoting their high penetration. Table 3 briefly describes overview of research work done on transethosomes for transdermal delivery of cardiovascular drugs.

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. Intercellular 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.

Table 3: Potential applications of nanoethosomes in delivery of cardiovascular drugs.

Drug	Excipients	Sophisticated Techniques used	Entrapment/size/PDI (polydispersity index)/ animal model	Key findings	Reference
Minoxidil	Phospholipon 90, ethanol, Phospho-tungstic acid	TEM, CLSM, HPLC, ^{31}P-NMR	83 ± 6%/ 153 ± 15 nm/-----/ Male albino mice	Prepared nanoethosomal formulation at 2% phosphatidylcholine and 30% ethanol showed rapid enhancement in transdermal permeability of compared hydroethanolic or phospholipid ethanolic solution of minoxidil	[10]
Valsartan	Phospholipon 90G, ethanol, Cholesterol	TEM, CLSM, HPLC	89.34 ± 2.54%/ 209 ± 15 nm/----/ Wistar albino rats	Results of study showed penetration of nanoethosomes in deeper skin layers compared to conventional liposomes and 3.03 times increase in bioavailability compared to oral suspension of valsartan	[45]
Valsartan	Phospholipon 90H, ethanol	SEM, HPLC	89.34 ± 2.54%/ 103 ± 5.0 nm/----/Wistar albino rats	Histopathological skin investigation showed dissolvation of intercellular lipids of epidermis by nanoethosomes promoting their high penetration	

Table 4: Investigations done on antiviral drugs delivered through nanoethosomes.

Drug	Excipients	Sophisticated Techniques used	Entrapment/size/ PDI (polydispersity index)/animal model	Key findings	Reference
Indinavir	Soya phosphatidylcholine, ethanol	TEM, SEM, HPLC	96.71 ± 1.4%/ 147 ± 4.5 nm/ 0.12 ± 0.03/ human cadaver skin	Nanoethosomes showed greater permeation of drug through human cadaver skin along with shortest lag time compared to conventional liposomes	[27]
Hepatitis B surface antigen–	Soya phosphatidylcholine, Span 80, ethanol	Flow-cytometric analysis, spectral bioimaging	----------	Nanoethosomes showed high internalizing capacity and immunogenicity compared to elastic liposomes following transcutaneous route	[48]
Acyclovir (ACV)/ Acyclovir Palmitate (ACV-C16)	Phosphatidyl choline, Cholesterol, ethanol	TEM, CLSM, HPLC	87.75 ± 12.2%/ 113 ± 12.1 nm/--------/ Kunming mice	ACV-C16 loaded nanoethosomes showed two times high drug entrapment and five times more skin permeation compared to ACV loaded nanoethosomes	[49]
Lopinavir	Phospholipon 90 H, cholesterol, ethanol	TEM, Fluorescence Microscopy, HPLC	79.6 ± 4.1%/ 112.8 ± 12.4 nm/ 0.131 ± 0.008/ Wistar rats	Fluorescence study revealed better disposition of ethosomal carrier in rat skin compared to niosomes; but, *in-vivo* extent of absorption was high in case of niosomal carrier system	[50]

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 nanoethosomal formulations 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.

Drug	Excipients	Sophisticated Techniques used	Entrapment/size/ PDI (polydispersity index)/animal model	Key findings	Reference
5-Aminolevulinic Acid (ALA)	Phosphatidyl ethanolamine, ethanol	Colorimetry, CLSM, HPLC	----------	CLSM study showed depth of penetration of nanoethosomes upto 80 µm in murine skin and penetration studies showed 26 folds increase in transdermal flux of nanoethosomes compared to plain ALA solution	[53]
Vitamin E acetate (a-tocopherol acetate, a-TA)	Decyltrimethyl, ammonium bromide, ethanol, cholesterol	Differential scanning calorimetry (DSC), membrane-fluorescence polarization technique, HPLC	67.51%/ 156.3 nm/---------/-------	Cationic nanoethosomes showed increase in stability, entrapment efficiency, and membrane rigidity after cholesterol addition	[54]
Paclitaxel	Phospholipon 90G, Absolute ethanol,	HPLC, TEM, Cell cycle analysis and apoptotic determination	82.00 ± 1.78%/ 240.0 ± 61.48 nm/ 0.145 ± 0.047/Adult human skin	Paclitaxel loaded nanoethosomes showed improved penetration capacity through stratum corneum epidermal membrane model and increased antiproliferative activity in squamous cell carcinoma model as compared to the free drug solution	[55]
Tacrolimus	Lipoid S 100, Absolute ethanol, cholesterol	TEM, HPLC	79.8 ± 1.6%/ 76.3 ± 0.5 nm/ 0.26 ± 0.01/ BALB/c mice	Tacrolimus loaded nanoethosomes showed higher encapsulation efficiency, lower vesicle size, and skin penetration compared to conventional liposomes with cholesterol	[56]
Testosterone propionate	Soybean phosphatidyl choline (PC), ethanol, cholesterol	TEM, DSC, HPLC, CLSM	92.7% ± 3.7%/ 156.5 ± 3.5 nm/ 0.100 ± 0.015/ Male albino mice	Prepared nanoethosomes showed high transdermal flux of 37.85 ± 2.8 µg/cm/hour and decreased lag time lag time of 0.18 hours across mouse skin. Nanoethosomes penetrated upto 260 µm in mouse skin	[57]
Apigenin	Lipoid S 75, propylene glycol, ethanol	TEM, HPLC	85.21 ± 3.97%/ 36.61 ± 1.78 nm/-------/ Sprague Dawley rats	Apigenin loaded nanoethosomes showed effective reduction of cyclooxygenase-2 levels in mouse skin inflammation induced by ultraviolet B (UVB) light compared to liposomes/ deformable liposomes	[58]
Psoralen	Lipoid S 100, ethanol	TEM, HPLC, Microdialysis	92.03 ± 8.95%/ 147.37 ± 17.04 nm/--------/ Sprague Dawley rats	Nanoethosomes showed high percutaneous permeability through abdominal rat skin and microdialysis study revealed 2.34 times higher area under the curve (AUC) compared to psoralen from the tincture	[59]
Ropivacaine	Soybean lecithin, Cholesterol, Ethanol	TEM, X-ray diffraction (wide angle X-ray scattering), DSC	68.92 ± 0.29%/ 73.86 ± 2.40 nm/ --------/ Kunming mice	Results of ex-vivo permeation studies showed high and rapid penetration of nanoethosomes in the skin, and histopathological studies showed weakening of the penetration barrier due to loosening of tight junction of corneocytes layers by impact of ethosomes	[60]
Vancomycin hydrochloride	Soy phosphatidyl choline, Cholesterol, Ethanol	Delivery of ethosomes in combination with iontophoresis	----------	Prepared nanoethosomes showed high electrochemical stability and cathodal iontophoresis of negatively charged nanoethosomes showed maximum transdermal flux (550 µg/cm^2/h) compared to ethosomes alone	[61]
Glimepiride	Phospholipon 90 G, Propylene glycol, Cholesterol, Ethanol	SEM, TEM, HPLC, CLSM	99.89% / 93 nm/----------/ Male Wistar rats	*In-vivo* study of ethosomes in human volunteers showed extended drug release behavior and lower maximum drug plasma level when used in the form of transdermal films	[62]

[64]. So, new classes of liposomes like deformable liposomes (DLs) and ethosomes (ELs) came into existence later on [65]. DLs are also recognised as 'Transferosomes' and they are the liposomes having addition of edge activator like span 60, span 25, span 80, tween 20, tween 60, tween 80, sodium deoxycholate, and sodium cholate [66]. Transferosomes show more effective transdermal drug delivery compared to CLs due to their high flexibility because of the presence of edge activators in them [67]. Transferosomes improve skin deposition of many drugs, however, they can't reach the stratum corneum deep enough [34]. Ethosomes (ELs), as we discussed earlier are composed of phospholipids, ethanol, and water and fluidization caused by ethanol may increase intercellular space between corneocytes and enhance their skin permeation. So, 'Transethosome (TELs)' represent a novel lipidic formulation that encompasses the advantage of both transferosomes and ethosomes. Transethosomes (TELs) show presence of high content of ethanol with edge activator or permeation enhancer

Table 6: Various patents related to nanoethosomes for transdermal drug delivery.

Title of patent	Brief description	Inventors	Patent Number
Ethosome preparation of male hormone medicaments and its preparation method	This invention describes the preparation technique of ethosomes loaded with male hormone used to treat various male diseases like male sterility, endocrine erectile dysfunction, and male climacteric syndrome	Guan Yan Min, Meng Shu, Li Jianxin, Dan	CN102406605 A
Progesterone ethosome, and preparation method and application there of	This invention describes a method of encapsulation of progesterone (0.1%-1%) in ethosomes for treatment of secondary amenorrhea, dysfunctional bleeding, and premenstrual syndrome	Zhang Shu, Deng Hong, Lin Huaqing, Zhang Xiaoling	CN102397255 B
Transdermal composition for treating pain	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	Moheb Maalawy	WO2015123750 A1
Preparation method of lidocaine ethosome	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	Liang Ju, Wu Wenlan, Li, Miao Juan, Wei Xuefeng, Chen Shan, Wang Xiao taro	CN102688194 B
Daptomycin ethosome preparation	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	Lee Chong, Liu Ha, Yanqi Kun, Wang Xiaoying, Chen Po	CN103006562 B
Phenasteroid gel preparation	This invention discloses preparation method of phenasteroid using 0.5-4% phospholipid and their dispersion in carbomer (0.25-1.5%) gel for topical application	Liang Wen- right, Rao Yuefeng	CN1555804 A
Bullatacin ethosome gel and preparation method thereof	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	Tan Jianping, Jiang Lixin, often calm, Zhou Zhiwen	CN102552147 B
Acyclovir ethosome and preparation method thereof	This invention discloses acyclovir loaded ethosomes with improved stability by addition of polyethylene glycol or chitosan for percutaneous administration	Wuxue Wen, Xiong Yan	CN102133183 B
Ethosomes preparation of antimycotics pharmaceutical and method for preparing the same	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	Liu Liping, Li Yimin, Shen Ming- high, six Jiang Hu, Yang Jin	CN101273971 A
Clotrimazole ethosomes for preventing and curing weaning rabbit dermatomycosis and preparation method thereof	This invention describes the composition and method of preparation of ethosomes loaded with clotrimazole having 3% of lecithin and 1% of clotrimazole by weight	Liu Man, Mou special, Liming Yong	CN104873465 A

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

Drug	Excipients	Sophisticated Techniques used	Entrapment/size/PDI (polydispersity index)/animal model	Key findings	Reference
Voriconazole	Lipoid S100, Cholesterol, Tween 80, Taurocholic acid sodium, Ethanol	TEM, HPLC	96.6 ± 2.7%/ 191.9 ± 41.5 nm/ ------/Male albino mice	Prepared transethosomes showed high elasticity, high *in-vitro* skin permeation, and high *in-vivo* skin deposition of voriconazole compared to nanoethosomes and conventional liposomes	[68]
Ketorolac Tromethamine	Phospholipon 90G, Sodium deoxycholate, Propylene Glycol, Ethanol	TEM, FT-IR	82.08 ± 4.5%/ 180 ± 70 nm/ -----/Male albino rats	Transethosomes showed 3 fold more elasticity compared to ethosomes and transethosomal gel 3 fold increase in transdermal flux compared to conventional ethosomes	[69]
Vitamin E/caffeine	Soybean phosphatidyl choline, Sodium cholate, ethanol	TEM, HPLC	For vitamin E- 76.689 ± 2.942%/ 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	For transdermal flux and stability, order obtained was: transethosomes (TE) > ethosomes (E) ≥ transfersomes (T) for both vitamin E and caffeine	[72]

like oleic acid [68]. Figure 4 shows formulation ingredients of transethosomes.

Method of preparation of transethosomes (TELs)

For the preparation of TELs, phosphoplipid, 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 ethosomes.

Research investigations proving transethosomes better compared to nanoethosomes

Garg et al. [70] prepared transethosomes loaded with piroxicam and compared them in gel form with liposomes, ethosomes, and transferosomes. Optimized transethosomes showed highest entrapment, elasticity, and improved stability compared to all other vesicular systems. Transethosomal gel showed highest drug permeation compared to other gel formulations. A comparative assessment between imiquimod loaded transethosomes and nanoethosomes for transdermal delivery was carried out by Ma et al. [71]. Transethosomes showed high accumulated drug (24.64 μg/cm^2) and local accumulation efficiency (6.70) compared to conventional ethosomes (14.45 μg/cm^2 and 3.93, respectively). Results of CLSM study revealed deeper skin penetration of transethosomes compared to conventional ethosomes [71]. Table 7 gives brief overview of research work done on transethosomes for transdermal drug delivery. No intellectual property rights (IPR) was found regarding transethosomes 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 transethosomes 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. Transethosomes 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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