Chapter 2

Transethosomes and Nanoethosomes: Recent Approach on Transdermal Drug Delivery System

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Additional information is available at the end of the chapter

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Abstract

In the past few decades, an emerging drug delivery system that came into light is transdermal drug delivery system. It has become the talk of the town in the field of drug delivery because of its better and easy accessibility. Though it is one of the attractive routes, transport of drug through the skin has remained a challenge. To overcome the challenge, vesicular system has been adopted so as to have better skin permeation of bioactive agents. Vesicular system like liposome has shown inefficiency to cross the layers of skin. Then transethosomes and nanoethosomes are employed for delivering drug into the deeper layer of skin. Nanoethosomes and transethosomes have same composition that is water, ethanol and phospholipid. Transethosome contains edge activator additionally. Due to the presence of ethanol and edge activator, it displayed enhanced skin permeation. Vesicular system gives a better patient compliance, being a non-invasive method of drug administration. In this chapter, we attempted to provide brief information about methods of preparation, characterization and pharmaceutical uses of nanoethosomes and transethosomes.

Keywords: transdermal, transethosomes, nanoethosomes

1. Introduction

In last few decades many significant advancement in the field of drug delivery technology have been made. This advancement took place as there was no remarkable growth in developing new drug entities. Drug delivery emerged as a branch of science which comprises of

biopharmaceutics and pharmacokinetics. Drug delivery technology is accepted as it imparts therapeutic as well as economical values to the health care products. The above said characteristic of drug delivery technology helps the old drugs to stand the competition of growing market. Drug delivery enhances the efficacy of drugs through controlled release by considering the factors like carrier system, route of administration and target of drug action. Drug delivery system improves patient compliance, therapeutic index and bioavailability [1].

In present scenario researchers are working on delivering the active pharmaceutical ingredient through transdermal route but still the effective delivery of drug through the said route is challenging. Transdermal drug delivery system originated in the year 1950 in U.S. In transdermal system drug permeate from the outer layer of skin to the blood stream through various layers of skin [2]. Transdermal drug delivery system is advantageous over traditional method. Drug administered through transdermal drug delivery system does not pass through GI Tract so are not exposed to degradation in the gut. As transdermal system delivers drug directly into blood stream through layers of skin, so drugs bypasses pre-systemic metabolism. Transdermal system is most suitable for drugs with short half life and narrow therapeutic range. The system helps in maintaining controlled level of plasma, in case of toxicity drug administration can be easily stopped. Few disadvantages are also attached to this system, such as drugs requiring blood volume cannot be administered [3–7]. Due the patches and the adhesive used in it, few recipients may experience skin irritation. For some section of patients it may not be economical. In the process of developing transdermal drug delivery system few basic areas has to be considered such as drug's bioactivity, the characteristics of skin, formulation employed, adhesive to be used and the design of the system. Permeation of drug through skin is dependent on factors like structure of skin and properties associated with it, the physicochemical property of the penetration molecule in relationship with the skin and lastly the system that carries the drug to the skin for penetration [8–10].

For overcoming the limitation of barrier function of skin some methods are undertaken such as iontophoresis, sonophoresis, electrophoresis along with micro-invasive techniques, vesicular system and employment of permeation enhancers [11, 12]. Transdermal system of drug delivery transports bioactive agents directly into blood stream. Transdermal system has many benefits like it bypasses first pass metabolism, it ensures better patient compliance and with this system there is mere chances of any kind of tissue injury. Currently vesicular system is mostly studied approach for transdermal drug delivery system. Vesicular system contains vesicles which are composed of colloids with hydrophilic and amphiphilic groups. Hydrophilic part makes the core and it is guarded by amphiphilic part in a bilayer effusion. The vesicular system act as a carrier for drugs which may be hydrophilic, lipophilic or amphiphilic. The vesicles have the ability of encapsulating the said drug types. Factors such as size, lamellarity, thermodynamic phase, surface charge are determinant of the efficiency of vesicular system as carrier [13].

Presently various type of vesicular carrier systems are available such as liposomes, ethosomes, transferosomes, niosomes, transethosomes etc. (**Figure 1**). All of them have their particular merits and demerits. There is not a single vesicular carrier that can fulfill all the criteria of drug delivery. In this chapter we are mainly focus on transethosomes and nanoethosomes vesicular carrier system.

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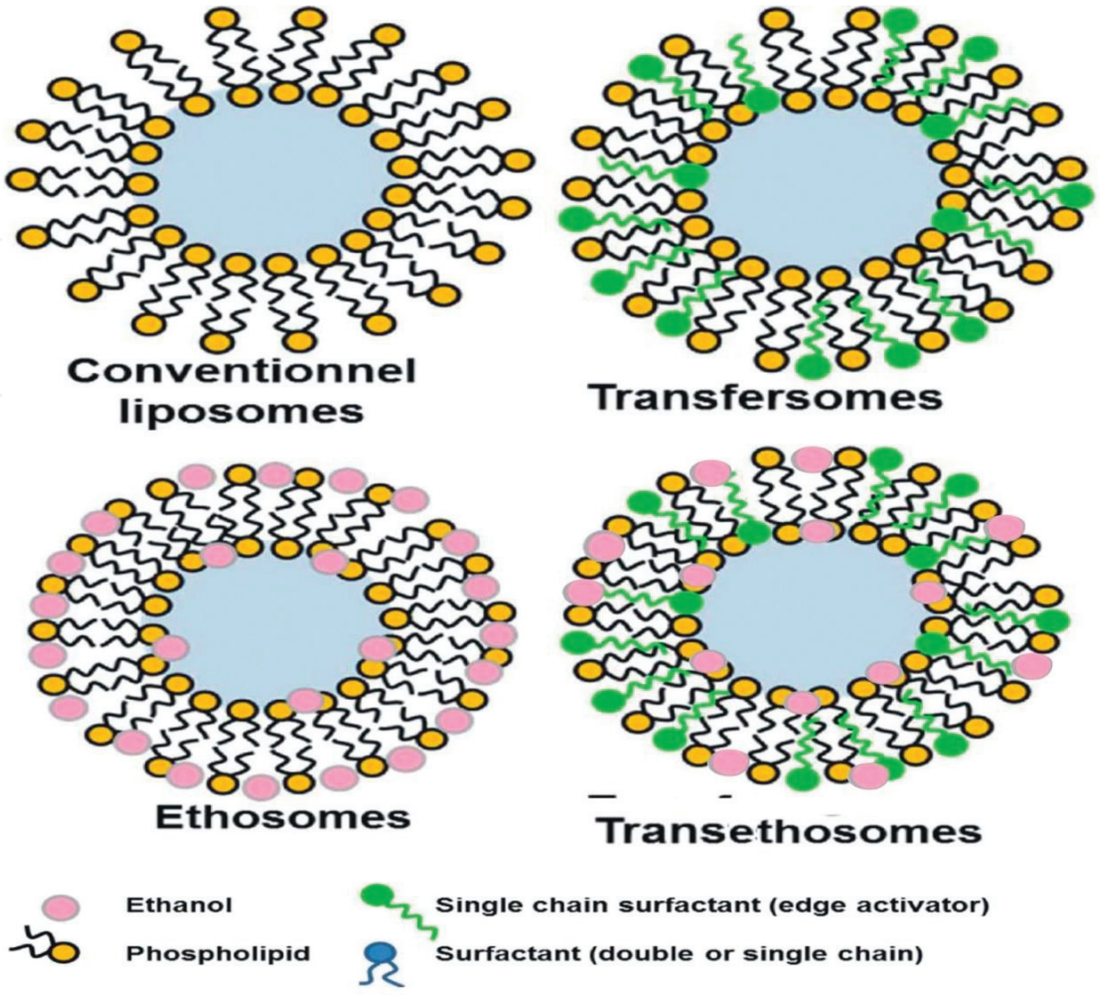

Figure 1. Schematic illustration of a panel of lipid-based nanocarrier transdermal delivery systems.

2. Transethosomes

Liposomes, which are conventional lipid-based vesicular system, are not capable of crossing the stratum corneum. As they exhibit low permeation into deeper layer of skin, eventually they get accumulated on superior layer of stratum corneum. To improve the skin permeation, liposomes are added with edge activators like Span 80, Span 25, Tween 80, sodium cholate and are named as transferosomes. Transferosomes enhances skin permeation due to their deformable ability but they are unable to penetrate deep into the stratum corneum. Another vesicular system called ethosomes is also available. Ethosomes are composed of phospholipid, ethanol and water (**Table 1**). Due to presence of ethanol the intercellular space between the corneocytes increases which increase the permeation [14–16].

Transethosomes are the combination of transferosome and ethosomes. Transferosomes shows both the quality of becoming deformable and skin permeation. This vesicular system was introduced in the year 2012 by Song et al. Transethosomes can be taken by topical as well as

S. No.	Additives	Liposomes	Ethosomes	Transethosomes	Examples	Uses
1.	Phospholipid	Present	Present	Present	Soya phosphatidyl choline	Vesicle forming component
2.	Polyglycol	Absent	Present	Present	Propylene glycol	Skin penetration enhancer
3.	Alcohol	Absent	Present	Present	Ethanol	Softness for vesicle membrane
4	Cholesterol	Present	Present	Present	cholesterol	Stability provider to vesicle membrane
5.	Vehicle	Present	Present	Present	Carbopol D934	Gel former
6.	Surfactant	Absent	Absent	Present	Sodium cholate	Edge activator

Table 1. Composition of different lipid vesicular carrier system.

systemic route. Drugs ranging from low molecular weight to high molecular weight can easily be entrapped by this system. As the bioactive agent is protected due to encapsulation, so it releases its content in a very slow and gradual manner. Due to their biodegradable and biocompatible nature, it shows highly efficient entrapment ability. Its preparation does not require any undue pharmaceutical additives and no tedious process is involved in it [15–17].

3. Nanoethosomes

Ethosomes are non-invasive carriers that assist the bioactive agents to penetrate deep into the layers of skin or into the blood stream [18]. This is a modified version of liposomal carrier system. They contains lipid vesicle along with water and ethanol. Ethosome was developed in the year 1996 by Touitou [19]. Ethosomes are known to release the entrapped bioactive agents in a constant and gradual manner. Ethosomes comes in various size. Ethosomes of nanometer size are called nanoethosomes. Presence of high content of ethanol imparts a negative charge on surface of vesicles which promotes reduction of its size [20]. Due to smaller size, nanoethosomes easily penetrate through the intercellular space [6].

4. Advantages and disadvantages of vesicular carriers

Many vesicular formulations for drug administration through parenteral, topical as well as oral route have been developed. Vesicular drugs provides advantages like convenience, safer way of drug administration and most importantly it provides a protection for the active constituent in in-vivo condition from premature degradation. In addition to the said advantages vesicular carriers makes it possible to release encapsulated molecule in sustained and controlled manner. Due to this pattern of release it becomes easy to ensure targeted delivery of drug to the target tissues. Challenges like pre-systemic metabolism, frequent dosing, and variation in GI absorption of drug can be overcome by vesicular carriers. Vesicular carriers reduce the dosing frequency due to which the cost to the patient decreases and ensures better

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patient compliance. Vesicular carrier increases the bioavailability as it enhances the permeation of drug through biological carriers. Only disadvantage associated with vesicular carrier is that few patients reported symptoms of dermatitis.

4.1. Advantages and disadvantages of transethosomes

Advantages of transethosomes are that it provides semisolid dosage form for administration which gives a better patient compliance. Transethosomes ensures an enhanced permeation of drug through skin. It bypasses presystemic metabolism. Few disadvantages of transethosomes are like dermatitis, allergic reaction or skin irritation in some patients. Some product gets lost as it is transferred from alcoholic media to aqueous media [21, 22].

4.2. Advantages and disadvantages of nanoethosomes

Nanoethosomes as a carrier makes it possible to deliver large molecules like proteins. The material used to prepare nanoethosomes is non-toxic, so it is not at all harmful for the recipient. As it is easy to use, so it provides better patient compliance. As compared to methods like phonophoresis, iontophoresis it is simple method of drug delivery. Few disadvantages are also associated with nanoethosomes such as it does not provide a rapid bolus drug input. To gain entry into blood circulation the drug has to be soluble in both lipophilic and aqueous phase. Adhesives used in nanoethosomes may not adhere on the skin of every single patient. A particular molecular size of drug can be can be delivered by this system. It may not be economical for certain segment of patients [23–25].

S. No.	Carriers/method	Advantage	Disadvantage
1.	Liposomes	Phospholipid vesicle, biocompatible	Less skin penetration less stable
2.	Physical methods, e.g., iontophoresis	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer efficiency is low (less than 10%)
3.	Niosomes	Non-ionic surfactants vesicles	Less skin penetration easy handling but will not reach up to deeper skin layer
4.	Transferosomes and protransferosomes	More stable, high penetration due to high deformability, biocompatible and biodegradable, suitable for both low and high molecular weight and also for lipophilic as well as hydrophilic drugs and reach up to deeper skin layers.	None, but for some limitations
5.	Transethosomes	• Enhanced drug permeation through skin for transdermal drug delivery. • Raw material in the formulation is non toxic in nature. • More stable • The transethosomal drug is administrated in a semisolid form. • Avoidance of first pass metabolism • Biocompatible and Biodegradable	• Product loss during transfer from alcoholic and water media. • Skin irritation or allergic reaction on contact dermatitis. • Unsuccessful vesicle formation can Coalesce transethosomes.

Table 2. Comparison with other vesicular systems with their advantages and disadvantages.

4.3. Challenges of vesicular carriers

Though vesicular carriers are becoming popular day by day but due to chemical reactions like oxidation and hydrolysis of the phospholipid component of vesicular carrier, its stability is a matter of concern. The physical and chemical instability can be seen by events like leakage of encapsulated drug and change in vesicle size which takes place due to fusion and aggregation.

4.4. Comparison with different vesicular carriers

The comparison of nanoethosomes and transethosome with other vesicular carriers are described in **Table 2**.

5. Penetration mechanisms for ethanol-based vesicular carriers

5.1. Ethanol effect on skin

Nanoethosomes and transethosomes contains around 20–50% of ethanol [26]. The action of ethanol on the lipid layer is shown in **Figure 2** with comparison to the lipid layer where there is no ethanol present. **Figure 2B** is showing void space is created and filled with ethanol that in turn increased area per lipid molecule [27]. X-ray diffraction shows that the lipid bilayer has interdigitated Membrane leaflet which eventually leads to thinner membrane when ethanol comes in contact with the lipid bilayer. As the surface density of lipid decreases the bilayer gets thinner which leads to membrane distention. Due to presence of alcohol at the surface the change in membrane shape get accelerated. Chanturiya et al. experimentally proved that alcohol promote fusion of discontinuous membrane by breaking the single layer continuity. This study do not confirm about alcohol accelerating post fusion membrane distention [28]. The result that comes up from this demonstration is that presence of ethanol can bring about

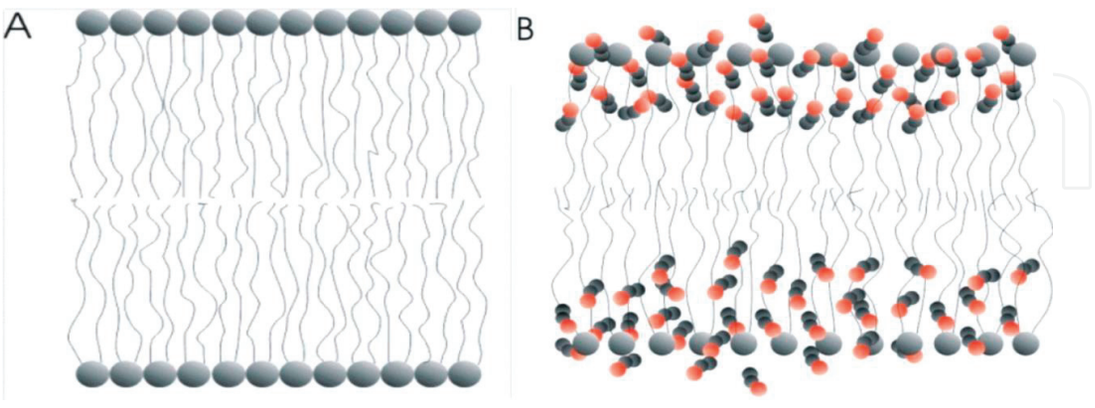

Figure 2. Schematic representation of ethanol's presence and absence on the bilayer lipid membrane: (A) representation of bilayer lipid membrane structure in the absence of ethanol; (B) presence of ethanol molecule is being represented as gray and red dots in the lipid bilayer membrane which partition into glycerol backbone and a hydrophilic head resulting in an increase in area per lipid molecule. (B) Also shows small leaflet interdigitation by representing the membrane relatively thinner as compared to (A) that shows the effect of presence of ethanol in (B).

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alteration in rate of change in shape by membrane in an exocytosis manner. In this demonstration the concentration of alcohol is quite higher as compared to that which is found in the blood in case of intoxication. Ethanol does accumulate in some region of body such as striatum, brain to an extent of three times of the level that is found in blood. Clearance from alcohol from striatum is relatively slower s compared to other region of body. This characteristic may affect chronic alcoholics during an event of binge drinking as the kinetics of neurotransmission may have an implication of the said characteristics [29].

5.2. Mechanism of skin penetration through vesicular carrier

Vesicular system assists in transdermal drug delivery of molecule either by enhancing penetration of free drug component or permeation is enhanced by the component of vesicles. In some cases the transdermal drug delivery takes place by intact vesicle penetration into the skin and then through it. Vesicle get adsorb and fused with the stratum and assist the transdermal drug delivery. Ethanol a component in ethosomes and nanoethosomes act as a great permeation enhancer as it fluidizes membranous lipid bilayer along with the lipid present in stratum corneum. Liposomes contain phosphatidyl choline which is tightly packed as compared to ethosomes or nano-ethosomes where phosphatidyl choline is loosely packed. Nano ethosomal vesicles have more flexibility as compared to liposomes. Stratum corneum composed of compactly packed phospholipid, when ethanol comes in contact with it disrupts the compact packing of phospholipid and fluidizes the lipid layer. This fluidization of lipid layer is the mechanism through which drug delivery by nano-ethosomes occurs. E. Touitou et al. has carried out DSC studies for ethosomal formulation which contains 30% ethanol, 5% phospholipid and liposomes were prepared without ethanol. An investigation is carried out by M.M.A Elsayed et al. to prove that the basic mechanism of skin delivery of drug is enhancement of permeation by ethanol and flexible nature of vesicles. The investigation focused on the in-vitro profile of drug outside the vesicles, drug inside the vesicle and drug on both side of vesicle. Out of the four the formulations the drug which was present inside the vesicular carrier displayed enhanced permeation then the remaining two formulations. From this it can be concluded that presence of ethanol is not major factor of permeation, if it would have been the case then drug outside the vesicle would have shown better permeation. This also suggests that the deformable nature of vesicle assist in enhanced penetration and drug delivery of drug. Godin and Touitou [30] proved vesicle adsorption to the skin and fusion of vesicular layer with stratum corneum of skin. It is observed that the drug present in liposomal preparation is unable to penetrate into skin although the preparation gets adsorbed to the membrane. In case of ethosomal preparation the ethanol present in it gets intercalated on lipid present in stratum corneum which results in increase in membrane permeability. Ethosomes are flexible and after fusion with the membrane they successfully deliver the drug inside the cells [31]. The main parameter that defines the movement of transfersomes across the cutaneous is extent of flexibility and partition coefficient. The passage of transferosomes through the stratum corneum layer is governed by hydration force. The fluid content of stratum corneum is lower than other layer of skin due to which fluid gradient created. Cevc et al. explained the passage through skin by osmotic strength theory [14]. The active ingredient can easily release in stratum corneum and diffuse through different layer of skin and reaches blood circulation. Transferosomes pass through the intercellular space of stratum corneum by

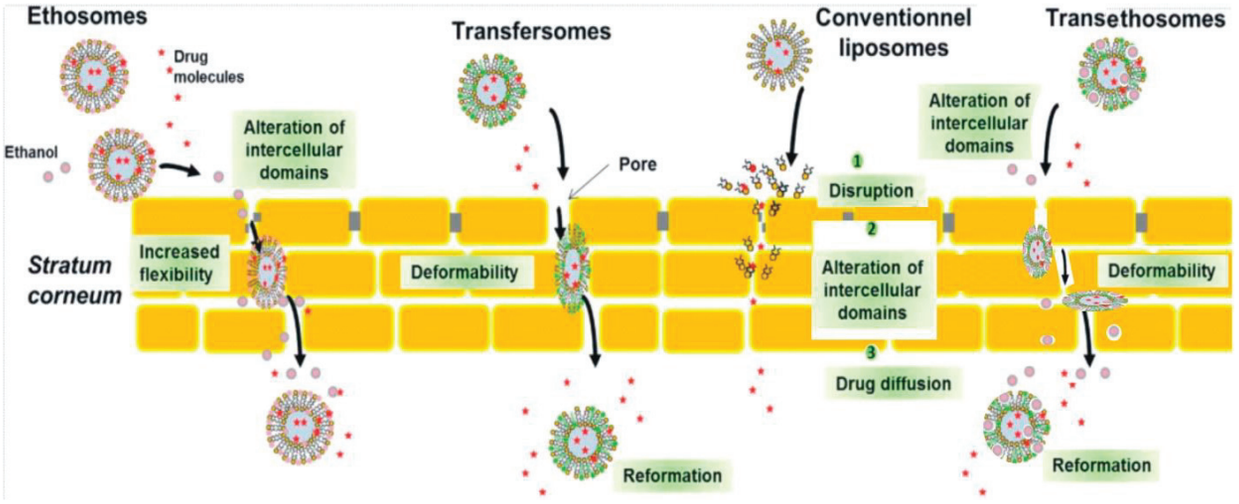

Figure 3. Schematic representation of the main permeation mechanisms of lipid-based vesicles.

deforming itself. No studies reported that transferosomes can permeate through the skin without altering its shape. The vesicular carrier transethosome makes its pathway through the stratum corneum into the deep skin layer by adhering to lipid lamella after interacting with the disturbed layer of stratum corneum. The presence of ethanol and edge activator in vesicles enhances its flexibility and fluidity and due this kind of elastic nature, vesicles can easily pass through narrow intercellular pathway. The permeation mechanism of different type of vesicular carriers is explained in **Figure 3**.

6. Method of preparation

There are four methods of preparation for nanoethosomes and transethosomes which are explain in here.

6.1. Preparation by cold method

In this method, ethanol is taken and to it phospholipid is added then by constant stirring it is dissolved at room temperature. Then to this solution propylene glycol is added and stirred vigorously. Then the solution mixture is heated up to a temperature of 30°C. Simultaneously on other hand water is being heated with up to a temperature of 30°C. Water and ethanol both solvents are used so that drug according to its affinity can dissolve in either of them. Stirring is continued to next 5 minutes. Then the suspension obtained is cooled down at maintained at room temperature. In the final step, by employing methods like extrusion or sonication, the vesicle size can be adjusted. Then the prepared ethosomal vesicles are kept in refrigerator for storage [32, 33].

6.2. Preparation by hot method

In hot method, a dispersion of phospholipid and water is subjected to heating at a temperature of 40°C. The step of heating at the same temperature is continued till colloidal solution is

formed. Simultaneously in another vessel two more solvent namely glycol and ethanol is added and heated at a 40°C. When both mixtures steadily attain a temperature of 40°C then to the aqueous phase the organic phase is added. Then stirring is done up to next 5 minutes and the obtained vesicle suspension is cooled down to room temperature. Depending on the ability and affinity of drug to bind with hydrophilic and hydrophobic solvent, it either gets dissolved in water or in ethanol. The required size of ethosome vesicles can be easily prepared by using methods like extrusion or sonication [6].

6.3. Preparation by classical method

Here in this method, a mixture of ethanol, active medicaments and phospholipid is taken in such way that the active medicament and phospholipid get dissolved in ethanol. Then the solution mixture is heated by using a water bath at a temperature of about 30 ± 1°C. In the next step to solution mixture double distilled water is added with continuous stirring at a speed of 700 rpm. Then with the help of hand extruder, the obtained vesicles are homogenized for three cycles using polycarbonate membrane [34].

6.4. Preparation by mechanical dispersion method

In this method a mixture of chloroform and ethanol is taken in a round bottom flask (RBF). To the round bottom flask soya phosphatidylcholine is added and made to dissolve in the chloroform and ethanol mixture. By using rotary vacuum evaporator organic solvent is removed. This step is carried out at a temperature that is above the lipid transition temperature. The main purpose of maintaining that temperature is that at the said temperature a thin lipid film gets deposited on the surface of a round bottom flask. Then the round bottom flask is kept overnight so that trace of solvent can be obtained from the lipid film that got deposited on the round bottom flask. Then hydroethanol hydration is being done by simply rotating round bottom flask at the required temperature by employing different concentration of drug mixture [35].

7. Evaluation parameters of vesicular carriers

7.1. Morphology

Morphology defined as study of shape and size of vesicular carriers. Generally vesicular carriers are regular spherical in shape and they are physically soft and flexible and core is enclosed. On the basis of formulation, vesicular carrier may be small, unilamellar or multilamellar. With the help of microscope morphology of vesicular carrier is studied. As most of the vesicles are nano-sized so to view the morphology scanning electron microscopy is used. In addition to identification study, morphology also explains the detection the pattern of packing of particles and aggregation [36].

7.2. Particle size and size distribution

As smaller size increases the efficiency of drug delivery to the desired site, vesicles in the vesicular carriers are of nanometer to micrometer range. Uniform size is major factor to

consider and it can be achieved depending on instrumentation and formulation procedures. Nanosizers are employed to measure the size and size distribution of vesicular carriers. Photon correlation spectroscopy is also used for determination of particle size [18].

7.3. Zeta potential

Distribution of charge on the surface of vesicular carrier is expressed by zeta potential. The presence of charge on the surface of nanoparticle is a major determinant of stability of the product. The presence of negative and positive charge on vesicular carrier depends upon the excipient used in the formulation. Zeta potential can be defined as the degree of electrostatic repulsion and attraction in colloidal dispersion. Zeta potential provides information regarding every components of formulation and interaction among them. It also gives information regarding surface chemistry. Zeta potential is determining factor for stability of colloidal dispersion system. It also determines the interaction between vesicles and membrane [37, 38].

7.4. Drug content

To ascertain whether the preparation content the required active ingredient in required amount in the vesicles, the vesicles are lyses so that the content is released. The released content is put into the solution then the solution is subjected to spectrophotometric analysis or chromatographic assay. Lysis of vesicles is done by solvents like isopropylalcohol, methanol, etc. [39].

7.5. Loading capacity, encapsulation efficiency, and vesicle yield

To have proper information about the drug that is actually trapped in the vesicles, entrapment is determined. For determination of entrapment, from the formed vesicles unentrapped drug is separated. Separation is done either by elution method using elution technique by the help of suitable column or by simply by the method of ultracentrifugation. Centrifugation is supposed to be done in such a controlled speed and temperature so that the vesicles do not get ruptured during the process. At end of centrifugation the supernatant produced is separated from the vesicles. Then the vesicles are treated with solvent likes triton-X. 2-propanol so that it get lysed then drug content is subjected to UV vis spectroscopy, the amount of drug entrapped is calculated [40]. The encapsulation efficiency (EE), Loading capacity (LC) and Vesicle Yield are calculated by the following formula's.

$$\text{EE}\,(\%) = \frac{\text{Ao} - \text{Au}}{\text{Ao}} \times 100 \qquad \text{LC}\,(\%) = \frac{\text{Ao} - \text{Au}}{W} \times 100$$

$$\text{Yield}\,(\%) = \frac{\text{Av}}{\text{Ao} + W} \times 100$$

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Where Ao is the initial amount of the drug used, Au is the nonencapsulated drug, W is the amount of lipid material used in the vesicular formulation and Av is the amount of vesicular carrier produced [41].

7.6. Drug release and biophysical interactions

Drug release is dependent on many factors such as the additives used in formulation and also the physiological condition at the site of administration. As we can consider when the particle size is relatively larger and the vesicular carriers the non-flexible then their permeation across the biological membrane is hampered. Every single body part possess its particular pH, if the formulation that has to be administered in that area is in that pH range then physiological functioning is not affected but in case the formulation has pH different from the physiological pH of concerned area then physiological condition would get affected. pH can cause ionization of drugs that can result in inability of the drug to pass through the biological barrier [42, 43].

7.7. Stability

The stability of the preparation can estimate by ensuring whether there is any change in vesicle size. When stability is concerned, it is believed that homogeneous preparation is more stable as compared to heterogeneous ones. Another way of determining the stability of preparation is to study its membrane stability and molecular arrangement by X-ray scattering or differential scanning method. Study of particle size is just considered as a quality control test not a test to ascertain the internal stability of preparation. This conclusion is made as it is seen in properly lyophilized liposome, where particle size is stable but additive like cryoprotectant interfere with lipid layer molecular arrangement in bilayer membrane [44].

8. Application of nanoethosomes and transethosomes

Ethosomes and nanoethosomes have enhanced efficiency as compared to liposomes. They give 65% better results in drug delivery as compared to liposomes as they easily crosses the human skin layers. Few bioactive agents are considered to investigate the efficacy of these vesicular systems which are described in **Tables 3** and **4** [45].

8.1. Transdermal delivery of anti-fungal bioactives

To ascertain the drug delivery efficiency of vesicular carrier system for anti-fugal drugs, Bhalaria et al. conducted an experiment where they took fluconazole and prepared nanoethosomes from them [46]. Then they took that nanoethosomal preparation and checked their efficacy in cutaneous candidiasis patients. This experiment showed positive results as the nanoethosomal preparations displayed a better clinical efficiency as compared to liposomal preparation, hydroethanolic solution and the marketed preparations of same drug. Girhepunje et al. conducted similar experiments taking ciclopiroxolamine ethosomes. Here he considered entrapment and optimized size and result of the experiment showed that due to presence of

Drug	Excipients	Sophisticated techniques used	EE (%)	Size/PDI	Animal model	Key findings
(1) Anti-fungal drugs						
Amphotericin B	Soya phosphatidylcholine, 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
Clotrimazole	Soya 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* anti-fungal activity compared to ultradeformable liposomes; along with high zone of inhibition compared to marketed formulation
Clotrimazole	Cavamax (W6, W7, and W8), propylene glycol, ethanol, triethanolamine, iso-propylmyristate	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 anti-fungal activity against *Candida albicans* and *Aspergillus niger* compared to normal ethosomal gel
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
(2) Cardiovascular drugs						
Minoxidil	Phospholipon 90, ethanol, phosphotungstic	TEM, CLSM, HPLC, 31P-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
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

Drug	Excipients	Sophisticated techniques used	EE (%)	Size/PDI	Animal model	Key findings
(3) Antiviral drugs						
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
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
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
(4) Anti-inflammatory drugs						
Triptolide	Dipalmitoylphosphatidyl 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
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
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
Ketoprofen	Soya phosphatidyl choline, cholesterol, ethanol	TEM, CLSM, HPLC	78.7 ± 4.9	120.3 ± 6.1 nm/–	Adult Chinese	Nanoethosomal formulation showed high transdermal flux and high *in-vitro*

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Drug	Excipients	Sophisticated techniques used	EE (%)	Size/PDI	Animal model	Key findings
					female skin	penetration compared to hydroethanolic solution of drug through human skin
(5) Miscellaneous bioactive materials						
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
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
Tacrolimus	Lipoid S 100, absolute ethanol	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
Paclitaxel	Phospholipon 90G, absolute	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
5–Aminolevulinic acid (ALA)	Phosphatidyl ethanolamine, ethanol	Colorimetry, CLSM, HPLC	—	—	—	CLSM study showed depth of penetration of nanoethosomes up to 80 μm in murine skin and penetration studies showed 26-folds increase in transdermal flux of nanoethosomes compared to plain ALA solution

Table 3. Applications of nanoethosomes for transdermal delivery of various classes of drugs.

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Drug	Excipients	Sophisticated Techniques used	EE (%)	Size/PDI	Animal model	Key findings
Voriconazole	Lipoid S100, cholesterol, Tween80, 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
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
Vitamin E	Soybean phosphatidyl choline, sodium cholate, ethanol	TEM, HPLC	76.689 ± 2.942	154.73 ± 1.89 nm/ 0.428 ± 0.020	Pig ear skin	For transdermal flux and stability, order obtained was: transethosomes (TE) > ethosomes (E) ≥ transferosomes (T) for vitamin E
Caffeine	Soybean phosphatidyl choline, Sodium cholate, ethanol	TEM, HPLC	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) ≥ transferosomes (T) for caffeine
Griseofulvin	Phospholipon 90G, Carbopol 980 NF,	TEM, HPLC fluorescence microscopy, reverse phase	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

Table 4. Applications of transethosomes for transdermal delivery of some miscellaneous drugs.

45% ethanol there is higher level of entrapment and vesicles formed are of optimized size [47]. **Table 3** provides information regarding few of the researches that has been conducted on nanoethosomal drug delivery of anti-fungal class of drug.

8.2. Transdermal delivery of anti-inflammatory bioactives

To have an idea about the efficacy of ethosomal preparation in delivering anti-inflammatory drug Paolino et al. conducted an investigative experiment on few human volunteers taking ethosomes entrapped with ammonium glycyrrhizinate. The ethosomal preparation which was having an ethanol content about 45% and having a lesser lecithin content gave better results both in *in-vitro* and *in-vivo* consideration. In case of in vitro it showed enhanced percutaneous

permeation, better tolerability of skin, and in vivo consideration showed enhanced anti-inflammatory activity in human volunteers. Nanoethosomes being smaller in size and having higher content of ethanol displayed better entrapment efficiency. Brief information has been given in **Table 3** regarding transdermal delivery of anti-inflammatory drug [48].

8.3. Transport of drug with action on cardiovascular system

To ascertain the extent of delivery of cardiovascular drug from transdermal system of drug delivery Touitou et al. investigated the efficiency of nanoethosomes filled with minoxidil. The nanoethosomes that were prepared with 2% of phosphatidylcholine and 30% of ethanol displayed a relatively increased permeability when compared with the formulation of hydroethanolic and phospholipid minoxidil. In another experiment Ahad et al. took valsartan and investigated the penetration capability of the nanoethosomes containing valsartan in the skin of Wistar albino rats. The results were amazing as there was increased penetration into deeper layers of skin when compared with liposomal preparation of same drug and it also shows increased bioavailability when compared with oral route of administration. In addition to this Bhosale and Avachat documented an increased antihypertensive action in Wistar albino rats when given transdermally as compared with oral administration of same [49]. **Table 3** shows role of nanoethosomes in antihypertensive drug delivery.

8.4. Transport of bioactive agent with antiviral activity

Nanoethosomes of lamivudine is been prepared by Jain et al. to evaluate the efficacy of transdermal delivery of antiviral drugs. The nanoethosomal preparation showed 25 times better penetration of drug through as compared to a simple solution. T-lymphocytes showed increased uptake ability for nanoethosomes compared to free drug solution [33]. Few more examples regarding transdermal drug delivery of antiviral drug is given in **Table 3**.

8.5. Delivery of miscellaneous bioactives

Nanoethosomes were compared with hydroethanolic and liposomal preparation, for that Dayan & Touitou prepared a nanoethosomes that was loaded with trihexyphenidyl HCl and result that came were really great as nanoethosomes showed better transdermal permeation than both the above mentioned preparation [50]. The penetration of nanoethosomes was deeper as compared to conventional liposomes. Dubey et al. studied melatonin loaded nanoethosomes in cadaver skin of human and are compared with liposomes. Result showed high mobility of drug through skin in case of nanoethosomes. **Table 3** provides brief information about nanoethosomes for delivery of various types of drugs [35].

9. Patent related to nanoethosomes

First patent related to nanoethosomes granted in 1986 by Prof. Elka Touitou of Hebrew University School of Pharmacy, Jerusalem. Descriptions of few patents regarding transdermal drug delivery of nanoethosomes are given in **Table 5.**

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, MengShu, 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 Xiaotaro	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, YanqiKun, Wang Xiaoying, Chen Po	CN103006562 B
Phenasteride 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, RaoYuefeng	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 anti-fungal drug containing 1–8% phospholipid, 20–45% ethanol, and 40.9–78.9% of water	Liu Liping, Li Yimin, ShenMing- high, six Jiang Hu, Yang Jin	CN101273971 A

Title of patent	Brief description	Inventors	Patent number
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 5. Description of patents related to nanoethosomes for transdermal drug delivery.

10. Conclusion

The work presented here is an overview on one of the current novel drug delivery device named as vesicular carrier. For researchers in the field of novel drug delivery vesicular carrier has become an area of interest which makes the future of vesicular carrier application bright in field of therapeutics and drug delivery. As vesicular carrier has an inert ability to better factors like solubility, penetration, uptake as well as it provides a better carrier facility to ensure the stability of various types of drugs and proteins, this makes it bankable delivery system of bioactive agents. In addition to this vesicular system is compatible with both hydrophilic as well as hydrophobic drug and easy encapsulate them. It also encapsulate herbal bio agent and ensure their stability. These said characteristics makes vesicular carrier system a promising option in the field pharmaceutical nanotechnology and herbal medicines. Vesicular carrier system has few thing as drawbacks like instability, relatively smaller short shelf life or difficulty to reach the target. All the drawbacks can be overcome by few techniques like providing appropriate storage condition to maintain stability, giving a polymeric coating to the preparation or conjugating the preparation with suitable ligands. To ascertain or assess the vesicular carrier stability and acceptability said parameters can be employed.

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References

[1] Almandil NB. Healthcare professionals' awareness and knowledge of adverse drug reactions and pharmacovigilance. Saudi Medical Journal. 2016;**37**:1359-1364. DOI: org/10.15537/smj.2016.12.17059

[2] Robert A, Freitas L Jr. Bioscience. In: Nanomedicine, Basic Capabilities. Vol. I. USA: Landes Bioscience; 1999

[3] Godin B, Touitou E. Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. Journal of Controlled Release. 2004;**94** (2–3):365-379

[4] Barry B. Breaching the skin's barrier to drugs. Nature Biotechnology. 2004;**22**(2):165-167

[5] Honeywell NPL, Bouwstra JA. Vesicles as a tool for transdermal and dermal delivery. Drug Discovery Today: Technologies. 2005;**2**(1):67-74

[6] Pandey V, Golhani D, Shukla R. Ethosomes: Versatile vesicular carriers for efficient transdermal delivery of therapeutic agents. Drug Delivery. 2015;**22**(8):988-1002. DOI: 10.3109/10717544.2014.889777

[7] Pirvu DC, Hlevca C, Ortan A, Prisada R. Elastic vesicles as drugs carriers through the skin. Farmácia. 2010;**58**(2):128-136

[8] Gregoriadis G, Florence AT. Liposomes in drug delivery. Clinical, diagnostic and ophthalmic potential. Drugs. 1993;**45**(1):15-28

[9] Sharma A, Sharma SU. Liposomes in drug delivery: Progress and limitations. International Journal of Pharmaceutics. 1997;**154**(2):123-140

[10] Schreier H, Bouwstra JA. Liposomes and niosomes as topical drug carriers: Dermal and transdermal drug delivery. Journal of Controlled Release. 1994;**30**(1):1-15

[11] Rai K, Gupta Y, Jain A, Jain SK. Transfersomes: Self-optimizing carriers for bioactives. PDA Journal of Pharmaceutical Science and Technology. 2008;**62**:362-379

[12] Regnier V, De Morre N, Jadoul A, Préat V. Mechanisms of a phosphorothioate oligonucleotide delivery by skin electroporation. International Journal of Pharmaceutics. 1999;**184**: 147-156

[13] Fahy E, Subramaniam S, Murphy R, Nishijima M, Raetz C, Shimizu T, et al. Update of the LIPID MAPS comprehensive classification system for lipid. Journal of Lipid Research. 2009;**50**(Supplement):S9-S14

[14] Cevc G. Transfersomes, liposomes and other lipid suspensions on the skin: Permeation enhancement, vesicle penetration, and transdermal drug delivery. Critical Reviews in Therapeutic Drug Carrier Systems. 1996;**13**(3–4):257-388. DOI: 10.4103/2231-4040.85524

[15] Honeywell-Nguyen PL, Bouwstra JA. The *in vitro* transport of pergolide from surfactant based elastic vesicles through human skin: A suggested mechanism of action. Journal of Controlled Release. 2003;**86**(1):145-156. DOI: 10.1016/S0168-3659(02)00415-7

[16] Trotta M, Peira E, Carlotti ME, Gallarate M. Deformable liposomes for dermal administration of methotrexate. International Journal of Pharmaceutics. 2004;**270**(1–2):119-125. DOI: 10.1016/j.ijpharm.2003.10.006

[17] Song CK, Balakrishnan P, Shim CK, Chung SJ, Chong S. A novel vesicular carrier, transethosome, for enhanced skin delivery of voriconazole: Characterization and *in vitro/in vivo* evaluation. Colloids and Surfaces. B, Biointerfaces. 2012;**92**:299-304. DOI: 10.1016/j.colsurfb.2011.12.004

[18] Mbah CC, Builders PF, Attama AA. Nanovesicular carriers as alternative drug delivery systems: Ethosomes in focus. Expert Opinion on Drug Delivery. 2014;**11**(1):45-59. DOI: 10.1517/17425247.2013.860130

[19] Touitou E. Composition of applying active substances to or through the skin. International Patent: International Publication Number US 5716638. 1996

[20] Lopez-Pinto JM, Gonzalez-Rodriguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. International Journal of Pharmaceutics. 2005; **298**(1):1-12. DOI: 10.1016/j.ijpharm.2005.02.021

[21] Zhou Y, Wei Y, Liu H, Zhang G, Wu X. Preparation and in vitro evaluation of ethosomal total alkaloids of Sophora alopecuroides loaded by a transmembrane pH-gradient method. AAPS PharmSciTech. 2010;**11**:1350-1358

[22] Verma P, Pathak K. Therapeutic and cosmeceutical potential of ethosomes: An overview. Journal of Advanced Pharmaceutical Technology & Research. 2010;**1**(3):274-282

[23] Vijay Kumar SK, Parthiban S, Kumarsenthil PG, Mani Tamiz T. ETHOSOME-A new trends In vesicular approaches for topical drug delivery. Asian Journal of Research in Pharmaceutical Sciences and Biotechnology. 2014;**2**(1):23-30

[24] Shahwal V, Samnani A, Dubey B, Bhowmick M. Ethosomes:An overview. International Journal of Biomedical and Advance Research. 2011;**2**:161-168

[25] Patel S. Ethosomes: A promising tool for transdermal delivery of drug. PharmaInfo.Net. 2007;**5**(3):38-43

[26] Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomes—Novel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. Journal of Controlled Release. 2005;**65**:403-418

[27] Lauer AC, Ramachandran C, Leib LM, Niemiec S, Simon ND. Effect of ethanol on membrane order: Fluorescence Weiner, targeted delivery to the pilosebaceous units via studies. Annals of the New York Academy of Sciences. 1987;**492**:125-133

[28] Bergelson LD, Molotkovsky JM, Manevich YM, Touitou L. A clinical evaluation of a novel liposomal carrier specific fluorescent probes in studies of biological mem-for acyclovir in the topical treatment of recurrent herpesbranes. Chemistry and Physics of Lipids. 1985;**37**: 165-161

[29] Gillet A, Compère P, Lecomte F, Hubert P, Ducat E, Evrard B, et al. Liposome surface charge influence on skin penetration behaviour. International Journal of Pharmaceutics. 2011;**411**:223-231. DOI: 10.1016/j.ijpharm.2011.03.049

[30] Godin B, Touitou E. Critical Reviews in Therapeutic Drug Carrier Systems. 2003;**20**(1): 63-102

[31] Mezei M, Gulusekharam V. Liposomes—A selective drug delivery system for the topical route of administration. Life Sciences. 1980;**26**:1473-1477

[32] Touitou E. Composition for applying active substances to or through the skin. International Patent: International Publication Number US 5716638 1998

[33] Jain S, Tiwary AK, Sapra B, Jain NK. Formulation and evaluation of ethosomes for transdermal delivery of lamivudine. AAPS PharmSciTech. 2007;**8**(4):E111. DOI: 10.1208/pt0804111

[34] Manosroi A, Jantrawut P, Khositsuntiwong N, Manosroi W, Manosroi J. Novel elastic nano-vesicles for cosmeceutical and pharmaceutical applications. Journal of the Science Faculty of Chiang Mai University. 2009;**36**(2):168-178

[35] Dubey V, Mishra D, Jain NK. Melatonin loaded ethanolic liposomes: Physicochemical characterization and enhanced transdermal delivery. European Journal of Pharmaceutics and Biopharmaceutics. 2007;**67**(2):398-405. DOI: 10.1016/j.ejpb.2007.03.007

[36] Maestrelli F, Capasso G, Gonzalez-Rodriguez ML, Rabasco AM, Ghelardini C, Mura P. Effect of preparation technique on the properties and in vivo efficacy of benzocaine-loaded ethosomes. Journal of Liposome Research. 2009;**19**(4):253-260

[37] Radomska-Soukharev A. Stability of lipid excipients in solid lipid nanoparticles. Advanced Drug Delivery Reviews. 2007;**59**:411-418

[38] Severino P, Szymanski M, Favaro M, Azzoni AR, Chaud MV, Santana MHA. Development and characterization of a cationic lipid nanocarrier as nonviral vector for gene therapy. European Journal of Pharmaceutical Sciences. 2015;**66**:78-82

[39] Mbah C, Builders P, Nzekwe I, Kunle O, Adikwu M, Attama A. Formulation and in vitro evaluation of pH-responsive ethosomes for vaginal delivery of metronidazole. Journal of Drug Delivery Science and Technology. 2014;**24**(6):565-571

[40] Jain S, Sapre R, Tiwary AK, Jain NK. Proultraflexible lipid vesicles for effective transdermal delivery of levonorgestrel: Development, characterization, and performance evaluation. AAPS PharmSciTech. 2005;**6**(3):E513-E522

[41] Nikpoor AR, Tavakkol-Afshari J, Gholizadeh Z, Sadri K, Babaei MH, Chamani J. Nano-liposome-mediated targeting of antibodies to tumors: IVIG antibodies as a model. International Journal of Pharmaceutics. 2015;**495**:162-170

[42] Almeida AJ, Florindo HF. Nanostructures overcoming the nasal barrier: Physiological considerations and mechanistic issues. In: Alonso MJ, Csaba NS, editors. Nanostructured Biomaterials for Overcoming Biological Barriers. Cambridge, MA: RSC Publishing; 2012. pp. 117-132

[43] Salama AH, Aburahma MH. Ufasomes nano-vesicles-based lyophilized platforms for intranasal delivery of cinnarizine: Preparation, optimization, ex-vivo histopathological safety assessment and mucosal confocal imaging. Pharmaceutical Development and Technology. 2014;**21**(6):706-715. DOI: 10837450.2015.1048553

[44] Stark B, Pabst G, Prassl R. Long-term stability of sterically stabilized liposomesby freezing and freeze-drying: Effects of cryoprotectants on structure. European Journal of Pharmaceutical Sciences. 2010;**4**:546-555

[45] Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M. Ethosomesnovel vesicular carriers for enhanced delivery: Characterization and skin penetration properties. Journal of Controlled Release. 2010;**65**(3):403-418. DOI: 10.1016/S0168-3659(99)00222-9

[46] Bhalaria MK, Naik S, Misra AN. Ethosomes: A novel delivery system for antifungal drugs in treatment of topical fungal disease. Indian Journal of Experimental Biology. 2009;**47**(5): 368-375

[47] Girhepunje K, Pal R, Gevariya H, Behera A, Thirumoorthy N. Ethosomes: A novel vesicular carrier for enhanced dermal delivery of Ciclopirox Olamine. Der Pharmacia Lettre. 2010;**2**(1):360-367

[48] Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M. Ethosomes for skin delivery of ammonium glycyrrhizinate: *In vitro* percutaneous permeation through human skin and *in vivo* skin anti-inflammatory activity on human volunteers. Journal of Controlled Release. 2005;**106**(1–2):99-110

[49] Ahad A, Aqil M, Kohli K, et al. Enhanced transdermal delivery of an anti-hypertensive agent via nanoethosomes: Statistical optimization, characterization and pharmacokinetic assessment. International Journal of Pharmaceutics. 2013;**443**(1–2):26-38. DOI: 10.1016/j.ijpharm.2013.01.011

[50] Dayan N, Touitou E. Carriers for skin delivery of trihexyphenidyl HCl ethosomes vs. liposomes. Biomaterials. 2001;**21**(18):1879-1885. DOI: 10.1016/S0142-9612(00)00063-6