Nano-transethosomes: A Novel Tool for Drug Delivery through Skin

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ABSTRACT

Transdermal drug delivery has become a popular tool from last few years. It overcomes drawbacks which are encountered with the oral route. Though very few routes are as attractive as transdermal route, transport of drug through the skin is challenging. In order to overcome the challenges, researchers have found a system in which the drug is encapsulated into the vesicle and these vesicles can penetrate deeper into the skin to hit the target site. Hence, better skin penetration of bioactive agents can be achieved. Vesicular systems like liposomes, niosomes, ethosomes and Transferosomes tend to remain accumulated in the skin layers. Since Transethosomes have small particle size and can easily alter the shape of vesicle as compared with other vesicular systems; it can penetrate through the layers of skin. Hence, the drug encapsulated into Transethosomes can easily reach the target site. Transethosomes consist of ethanol, phospholipids along with an edge activator. Ethanol and edge activator help to enhance skin permeation of Transethosomes. Since it is a non-invasive technique, it improves patient compliance. It also increases drug entrapment efficiency. These vesicles can accommodate different variety of drugs such as anticancer, corticosteroids, proteins and peptides, analgesics.

Key words: Transdermal delivery system, Transethosomes, Vesicles, Skin permeation, Non-invasive, Entrapment efficiency.

INTRODUCTION

Oral route is most commonly used route of administration. It is most convenient to administer drugs by oral route but some oral formulations may have significant drawbacks like reduced bioavailability due to first pass metabolism, gastric irritation, unpalatable taste, etc. To overcome these difficulties, transdermal route has been tried having merits such as bypasses first pass metabolism. Transdermal formulation can show better bioavailability than oral route for drugs having high first pass metabolism. It has some limitations like drugs with higher molecular weight cannot penetrate the stratum corneum. Drugs with high or low partition coefficient have difficulty in reaching systemic circulation. Liposomes are used to deliver drugs in to the skin but they tend to remain in upper stratum corneum thus limiting the penetration of drugs. To improve drug delivery, novel lipid vesicles known as ultra-deformable vesicles (UDV) have been developed. Various types of UDV such as ethosomes, transferosomes and transethosomes are developed for administration of cosmeceuticals and pharmaceuticals. Transferosomes are elastic vesicular carriers in which edge activator (biocompatible surfactant) is incorporated in to lipid bilayer structure. Even after evaporation of water from the formulation, edge activator remains in the formulation. The major disadvantage with such formulation is that it is difficult to load hydrophobic drugs in these vesicles without compromising the elastic properties. That is why; ethosomes are prepared which eliminates the disadvantage of transferosomes. Ethosomes are vesicular carriers consisting of hydro-alcoholic phospholipids in which concentration of alcohol is high. The major disadvantage of
ethosomes is that it causes dehydration of skin due to evaporation of ethanol from the formulation as soon as it is applied on skin under non-occlusive condition. So tranethosomes are prepared which is a combination of both transferosomes and ethosomes. It has uneven spherical shape and it is highly elastic. It can easily encapsulate the drugs of both low molecular and high molecular weight.

**Comparison of Ethosomes, Transferosomes and Tranethosomes**

Table 1 below, shows the difference between ethosomes, transferosomes and tranethosomes. It is observed that Tranethosomes are comparatively better than other formulations as it has high drug entrapment efficiency compared to other formulations. It has the ability to alter the shape and to penetrate into deep layers of skin.

**Structure of Tranethosome**

Tranethosomes are lipid-based vesicles containing phospholipids, ethanol, edge activator (surfactant) and water as observed in Figure 1. Phospholipids (or non-ionic surfactants) play the role of carrier for delivering drug molecules into the skin. They can easily interact with stratum corneum, improve tissue hydration and merge with lipids of the stratum corneum. They contain a hydrophilic (polar) head as well as hydrophobic (non-polar) tail. Edge activator (biocompatible surfactant) is a bilayer softening agent. It is usually added to improve flexibility and permeability.

Alcohol is a primary character of the tranethosomal system which gives an unusual identity to it as a vesicular system. Ethanol deforms the layer of skin and leads to malleability and flexibility of these nano systems enabling them to penetrate inside stratum corneum through tiny openings due to fluidization. Water is the essential component as it helps to form bilayer when phospholipids are added and help in flexibility of system. When ethanol and edge activator are combined, it leads to rearrangement of lipid bilayer and it becomes more deformable such that it can penetrate deeper into the dermis.

**Composition of tranethosomes and their role**

The components of tranethosomes are categorized under ‘Generally Recognized as Safe’ (GRAS) listed substances. Commonly used excipients having GRAS status are mentioned in the Table 2.

**Methods of Preparation of Tranethosomes**

Tranethosomes are simple to prepare and easy to scale up without involvement of sophisticated equipment’s at both pilot plant and industrial level. Different methods are used to achieve small vesicular size and these vesicles are incorporated into gels or creams to increase skin penetration. The following are some of commonly used methods.

**Ethanol injection method**

This method has many advantages over other techniques as it is easy to prepare and scale up. Ethanol is used as organic solvent due to its harmless nature. This method is well explained in Figure 2.

![Figure 1: Structure of Tranethosomes.](image1.png)

![Figure 2: Ethanol injection method.](image2.png)
**Thin layer hydration method**
It is easy to prepare. High encapsulation of lipid and aqueous substances can be achieved. One major disadvantage is that it is time consuming so it gives difficulty in scaling up. This method is depicted in Figure 3. \(^{29,30}\)

**Cold method**
This method is usually used to form tranethosomes. Its ability is such that it can be used for thermolabile drugs that are sensitive to heat. It is easily scalable. \(^{31}\) This is well explained with the help of flow chart (Figure 4).

**Direct method**
The required quantity of phospholipids, edge activator and drug are dissolved in organic solvent. Aqueous solution is added to the organic solvent. Required mixture is homogenized for 5 to 10 min. Sample is then filtered. It is easy to perform and to scale up. \(^{32}\)

**Reverse phase evaporation**
It is effective method and has high encapsulation rate, up to 50\%. \(^{27}\) The method is explained in Figure 5.

**Optimization of formulation containing tranethosomes**
Various process variables which affect the preparation and properties of tranethosomes are concentration of drug, percentage of edge activator, percentage of lipid, percentage of ethanol. These are independent variables which can be optimized to achieve desired entrapment efficiency, flux rate, vesicle size and drug permeation. During the preparation of particular system, other variables are kept constant, example; concentration of drug and edge activators are kept constant, other variables are optimized to achieve target product profile. \(^{24,29}\)

**Anatomy of skin**
Skin is made up of three layers, that are; epidermis, dermis and hypodermis.

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**Figure 3: Thin layer hydration method.**

**Figure 4: Cold method.**

**Figure 5: Reverse phase evaporation.**

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<table>
<thead>
<tr>
<th>Table 2: Composition of Tranethosomes.</th>
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<tbody>
<tr>
<td>Composition</td>
</tr>
<tr>
<td>Phospholipids (2%– 5%)(^{24})</td>
</tr>
<tr>
<td>Edge activator(^{26})</td>
</tr>
<tr>
<td>Alcohol (30% - 40%)(^{13})</td>
</tr>
<tr>
<td>Water (qs 100)</td>
</tr>
</tbody>
</table>
Epidermis: It is the outer layer of skin and is made up of keratinocytes. It contains two types of epidermal layers i.e. Non-viable and viable epidermal layer

Non-viable epidermal layer is called Stratum corneum (horny layer): It is the exterior layer of skin. It is composed of closely packed lipid bilayers which are present between the corneocytes. It acts as major barrier for drug absorption. It prevents the foreign substance to enter into the body.

Dermis: This layer consists of matrix of connective tissues from where absorption of drug takes place. Hair follicles, sebaceous glands and sweat glands rise from the dermis to the outward layer of the skin which also takes part in the transport of drug.

Hypodermis: It consists of subcutaneous fat tissue which acts like a shock absorber for blood vessels and nerve endings. It is a protective and nutritive layer.

Mechanism of Drug Permeation: Major barrier for drug absorption is stratum corneum. Transport of drug through stratum corneum can be achieved by transferring the drug through three pathways namely intracellular, intercellular and follicular pathways. The transethosomes are able to cross stratum corneum by following two pathways.

Ethanol: When ethanol comes in contact with stratum corneum it disrupts the phospholipids and fluidizes lipid layer which is present in stratum corneum. It increases the intracellular space connecting the corneocytes which in turn increases the permeation and slowly releases the therapeutic agent into skin layers.

Edge Activator: It causes disruption of intercellular lipids and widens the hydrophilic pores of the skin. Through these pores, the drug is released gradually. This causes molecular interaction which then increases skin penetration.

Due to presence of both ethanol and edge activator, it increases fluidity and elasticity respectively of transeithosomes. As fluidity of lipid layer increases, there is reduction in its size. Due to elastic behaviour, the shape can be altered such that it can pass through the narrow regions of intercellular pathway. After passing through stratum corneum, it passes through viable epidermis and reaches the dermis. Mechanism of permeation of transeithosomes is explained in Figure 6.

Salient features of transeithosomes
1. It is highly flexible so it has high flux rate and high rate of skin permeation compared to other vesicular systems.

Advantages and disadvantages of transeithosomes

Advantages
1. It is a non-invasive technique.
2. It bypasses first-pass metabolism and avoids side effects like irritation to gastric mucosa, vomiting because of unpalatable taste.
3. It is more stable than other ultra-deformable vesicles
4. It can easily penetrate through skin layers.
5. Sustain release and control release can be possible with transeithosomes as a drug can be encapsulated into it.
6. It can be administered as semisolid dosage forms like gel or cream so as to have high patient compliance.

Disadvantages
1. Since ethanol is used in the formulation, it can cause skin irritation, an allergic reaction and also dermatitis.
2. When there is an incomplete formation of vesicles it can lead to coalescence of transeithosomes.

Characterization of transeithosomes

Vesicular Shape
Vesicular shape can be observed under Transmission Electron Microscopy (TEM). Sample is placed in carbon-coated copper grid to form thin film grid. It is negatively stained by phosphotungstic acid.
Kaur et al. observed that transethosomes were present in irregular spherical shape under TEM.\textsuperscript{49}

**Vesicular size and Zeta potential**

It can be recognized by Particle Size Analyzer and Light Scattering Technique. In light scattering technique, particles of different sizes can be scattered through light. The vesicular diameter can be recognized by photon correction spectroscopy or dynamic light scattering (DLS).\textsuperscript{50}

Zeta potential is the measure of electrostatic repulsion and attraction in colloidal dispersion. Zeta potential can also provide information regarding surface chemistry. It is the determining factor for firmness of colloidal dispersion system.\textsuperscript{51}

Results of particle size, zeta potential, PDI obtained through various studies are compiled in Table 3. As seen from results, the particle size of vesicles range from 80nm to 250nm. It was observed that the values of zeta potential were higher which increased stability of the vesicle due to higher electrostatic repulsion.

**Loading capacity (LC), Encapsulation efficiency (EE), Vesicle yields**

EE can provide the information regarding actual amount of drug trapped in the vesicle. The mini column centrifugation method can be used. Vesicular suspension is loaded with appropriate amount of drug which is then placed into mini column and the column is centrifuged. The eluted vesicles are collected from the column and observed under the microscope. Centrifugation is performed in such a way that speed and temperature are controlled. Because of this, vesicles do not get fractured during the process. In the last stage of centrifugation, the supernatant produced is separated from vesicles. Vesicles are treated with solvents like; triton-X 2-propanol, methanol in order to get lysed. The drug content is then analysed using UV visible spectrophotometry. The quantity of drug entrapped can be calculated using following formula.\textsuperscript{52,53}

\[
\text{Percentage drug entrainment} = \frac{\text{Amount of entrapped drug}}{\text{Total amount of drug}} \times 100
\]

\[
\text{EE}\% = \frac{\text{Ao} - \text{Au}}{\text{Ao}} \times 100
\]

\[
\text{LC}\% = \frac{\text{Ao} - \text{Au}}{\text{W}} \times 100
\]

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

\( \text{Ao} \) = Initial quantity of the drug used

\( \text{Au} \) = Non-encapsulated drug

\( \text{W} \) = Amount of lipid material which are used in the formulation of vesicles

\( \text{Av} \) = Amount of vesicular carrier produced

Based on literature, it was observed that small size of particle showed improved penetration of drug. Particle size of tranethosomes was found to be smaller than other vesicles.

**Phase transition temperature**

Phase transition temperature is studied to understand the release of drug from the vesicles. It can be identified using Differential Scanning Calorimeter (DSC). Each sample is analysed at a temperature range under a constant nitrogen stream. With the help of differential thermal curves, the samples are compared.\textsuperscript{20}

**Elasticity measurement**

It is the most significant property for efficient penetration of skin. Extrusion method is used to find elasticity of the vesicular bilayer. Vesicles are extruded through cellulose membrane filter of appropriate pore diameter by applying pressure. Dispersion of vesicles which are extruded are calculated by\textsuperscript{56}

\[
\text{E} = \frac{\text{J} \times (\text{rv} / \text{rp})^2}{2}
\]

\( \text{E} \) = Elasticity index of vesicle membrane

\( \text{J} \) = Rate of penetration through membrane filter

\( \text{rv} \) = Vesicle size after extrusion

\( \text{rp} \) = Pore size of membrane

Shaji et al. concluded that tranethosomal formulation had higher elasticity index compared with ethosomal formulation. Hence drug penetration through skin is found to be higher.\textsuperscript{54}

**In vitro drug release study**

The quantity of drug release can be studied using dialysis bag method. In this method, the tranethosome formulation is loaded in dialysis membrane. The loaded membrane is taken into conical flask containing buffer solution and is incubated. At given time intervals, aliquots are withdrawn and centrifuged by using mini

<table>
<thead>
<tr>
<th>Tranethosomal Drugs</th>
<th>Particle Size (nm)</th>
<th>Zeta Potential (mV)</th>
<th>% Entrapment Efficiency</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imiquimod</td>
<td>82.3 ± 9.5 nm</td>
<td>-29.0 ± 1.9</td>
<td>68.69 ± 1.7%</td>
<td>53</td>
</tr>
<tr>
<td>Ketorolac tromethamine</td>
<td>180 ± 70nm</td>
<td>-46.19 ± 13.3</td>
<td>82.08 ± 4.5%</td>
<td>54</td>
</tr>
<tr>
<td>Olmesartan medoxomil</td>
<td>222.6± 2.59 nm</td>
<td>-20.81± 0.34</td>
<td>58.54 ± 1.30%</td>
<td>55</td>
</tr>
</tbody>
</table>
column centrifugation. The free drug is assessed by suitable method. Kaur et al. found that sustained release of drug can be achieved over 24 h. This can reduce dosing frequency which leads to better patient compliance. Shaji et al. performed an experiment where they took ketorolac tromethamine as active ingredient and transethosomal gel was prepared which was compared with ethosomes. This experiment showed positive result as the gel displayed better skin penetration due to its elastic behaviour compared to theethosomes.

**In vivo skin deposition**

Skin disposition is carried out in animals like rats, mice to find the distribution of drug through various layers of skin after administration of transethosomal formulation. After 24 h of administration, the amount of drug deposited in the stratum corneum is measured with the help of fluorescence spectrophotometer. Confocal Laser Scanning Microscopy (CLSM) is used to visualize complete distribution of drug through various layers of skin.

Albash et al. concluded that the transethosomes overcome the barrier of stratum corneum and penetrate deeper within layers of skin.

**Ex vivo Skin permeation Studies**

Skin permeation is studied using fresh animal’s skin e.g. goat, rat. The skin sample is mounted on Franz diffusion cell containing phosphate buffer saline in the receptor compartment. On donor side of diffusion cell facing the stratum corneum the formulation is applied. Sample is removed from receiver compartment of cell at different intervals and at constant temperature. The sink condition is maintained and sample is analyzed using HPLC.

The cumulative amount of drug permeated through animal’s skin is calculated by

\[ Q_n = [C_1 V_r + \sum C_i V_i] \]

where:

- \( Q_n \) = Cumulative amount of drug per unit area.
- \( C_1 \) = Concentration of drug at each sample interval.
- \( V_r \) = Receptor volume of individual Franz diffusion cell.
- \( C_i \) = Concentration of drug at the \( i^{th} \) sample.
- \( V_i \) = Sampling volume.

**Applications of transethosomes**

**Delivery of Non-Steroidal Anti-Inflammatory Drugs (NSAIDS)**

NSAIDS are usually administered by oral route but it is associated with numerous gastrointestinal side effects. To overcome this problem, researchers tried delivering the drug through transdermal route using ultra deformable vesicles. Garg V et al. performed the experiment using active ingredient piroxicam which was then formulated into transethosomal gel. It was observed that, this formulation displayed greater stability and elasticity over other vesicular carriers.

Shaji et al. performed an experiment where they took ketorolac tromethamine as active ingredient and transethosomal gel was prepared which was compared with ethosomes. This experiment showed positive result as the gel displayed better skin penetration due to its elastic behaviour compared to theethosomes.

**Delivery of Antifungal drugs**

To find the drug delivery efficacy of transethosomes, for delivering antifungal drug, Verma et al. used Econazole Nitrate as active moiety; and compared econazole nitrate loaded transethosomal gel with marketed econazole nitrate transdermal cream. They found that transethosomal gel showed high ex vivo skin retention and high in vitro antifungal activity. Transethosomal gel releases the drug in the controlled manner which eliminates the cutaneous candidiasis.

**Delivery of Anticancer drugs**

Lei et al. conducted the experiments by dual loading of drugs in to transethosomal formulation to treat cutaneous melanoma. They selected two drugs which had synergistic action such as dacarbazine and tretinoin and which eliminated cytotoxic effect compared to the other formulations. Dual loaded transethosomes showed increase anticancer activity compared with single loaded drug. They found that enhanced penetration of skin can be achieved.

Shaji et al. concluded that encapsulation of 5-Fluorouracil into transethosomal gel showed improved deformability that increased skin penetration and deeper skin targeting was achieved compared with ethosomes.

**Delivery of peptide drugs**

As peptides are larger in size, they are unable to pass through stratum corneum. Hence, transdermal delivery of peptides is difficult.

Kim et al. performed an experiment by encapsulating palmitoyl pentapeptide into transethosomal formulation to improve transdermal delivery. They concluded that palmitoyl pentapeptide, when loaded into transethosomal formulation improved the flexibility which increased skin penetration.

**Delivery of Anti-hypertensive Drug**

Usually anti-hypertensive drugs are taken by oral route but some drugs have lower bioavailability due to first pass metabolism.
Albash et al. took olmesartan medoxomil as active ingredient and formulated a transethosomal formulation which showed increase in drug penetration through skin by transdermal route.\textsuperscript{55}

**Delivery of Anti-arthritis Drugs**

Song et al. conducted an experiment using the drug sinomenine hydrochloride. Transethosomes was loaded with Sinomenine hydrochloride which was then decorated with ascorbic acid to form antioxidant surface transethosomes. This showed enhanced transdermal permeability and drug disposition for oxidative stress of rheumatoid arthritis.\textsuperscript{54} Some more applications are compiled in the Table 4.

**Regulatory aspects**

Regulatory affairs of pharmaceuticals involve a blend of legislation and guidelines developed in order to maintain and approve safety, quality and efficacy of the drug products before it reaches to consumers. This means that development, production, importation, exportation and distribution of drug should be regulated to meet standard specifications. The manufacturer should ensure that tranethosomal formulation meets the desired specification. Excipients which are used by the researchers are clinically non-toxic and listed “Generally recognized as Safe” (GRAS). This GRAS listed excipients are mentioned in Table 2. The researchers have maintained stability at various temperatures that is $40 \pm 2^\circ C$, $25 \pm 2^\circ C$ and $4 \pm 2^\circ C$ at different time intervals. It ensures that size and drug entrapment does not change. The researchers have used the excipients within the prescribed range in ‘Inactive Ingredient Guide’. It is a record which has been prepared by FDA that comprises of a permitted excipients catalogue. This document provides information regarding excipients with a maximum value of dosage level by fastidious route of administration of dosage form.\textsuperscript{14,16} Table 5 shows the amount of maximum potency of the inactive ingredient which is approved by FDA. \textsuperscript{65} The tranethosomal formulations are prepared by using the excipients in prescribed limits.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Excipients</th>
<th>Method used</th>
<th>Entrapment/size/ PDI</th>
<th>Key findings</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol Hydrochloride (Non-selective β-blocker)</td>
<td>Lipoid S100, oleic acid, Rhodamine B, ethanol</td>
<td>Homogenization method</td>
<td>81.98 ± 2.9%/ 182.7 ± 5.4 nm/ 0.234 ± 0.039</td>
<td>When loaded in tranethosomes it showed higher stability, prolonged release of drug and excellent in-vitro skin permeation. Tranethosomal gel maintained an effective plasma concentration level compared to marketed oral tablet</td>
<td>58</td>
</tr>
<tr>
<td>Paeonol (Anti-inflammatory)</td>
<td>Soya phosphatidylcholine, Tween 80, ethanol, ultrapure water.</td>
<td>Ethanol injection method</td>
<td>85.5 ± 52%/122.5 ±7.5nm</td>
<td>Paeonol loaded tranethosomes showed increased stability and bioavailability than other vesicular system. This improves patient compliance.</td>
<td>28</td>
</tr>
<tr>
<td>Capsaicin (Anticancer)</td>
<td>Phospholipon 90G, carbomer, span 80, triethanolamine, sodium hydroxide, potassium dihydrogen phosphate, ethanol, methanol</td>
<td>Hydration thin layer method</td>
<td>84.85±1.15% / 174.9±2.02 nm/ 0.266±0.01</td>
<td>Tranethosomal gel increase penetration of capsaicin through skin than non tranethosome capsaicin gel.</td>
<td>32</td>
</tr>
</tbody>
</table>

**FUTURE PROSPECTS**

Of the current novel drug delivery systems, tranethosomal vesicular carriers have attracted attention of researchers. It makes the future bright, in
the field of transdermal drug delivery and therapeutics. It provides better carrier system to ensure the stability of different types of proteins and drugs. It is suitable for loading both hydrophilic as well as hydrophobic drugs. Various classes of drug such as antiviral, anti-diabetics, Anti-coagulant can be tried using transethosomes. Anticancer, drug combination can be administered with transethosomal delivery with minimum cytotoxicity and good skin permeation. Similarly, combination of other drugs can be administered as transethosomes to increase the efficacy of drug. As transethosomes are not commercially available, there is not much literature is available on clinical trials. Thus, transethosomes have lot of potential to use as the carrier for transdermal or topical drug delivery.

CONCLUSION

Some active moieties are unable to penetrate through skin barriers. Drug encapsulated into transethosomes passes the stratum corneum by intercellular as well as intracellular pathways. Transethosomes have ethanol and edge activator in its composition. Ethanol increases the fluidity of lipid layer and reduces the particle size. Edge activator helps in deformation and penetration into the skin pore. Due to both small particle size and deformability, transethosomes can penetrate through various layers of skin. Transethosomes can be used in treatment of cancer, especially skin cancer. Biomolecules being bigger in size can pierce deeper into skin as they are encapsulated in transethosome formulation. In the field of pharmaceutical nanotechnology and herbal medicine, these characteristics make the transethosome vesicle carrier system a promising tool. A wide scale of research is required in this field to make this carrier system a commercial success.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

TDDS: Transdermal drug delivery system; NSAIDs: Non-steroidal Anti-Inflammatory Drugs; UDVs: Ultra-deformable Vesicles; GRAS: Generally Recognized as Safe; TE: Transethosomes; TEM: Transmission Electron Microscopy; DLS: Dynamic Light Scattering;


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Transdermal route is the most promising tool in drug delivery system as it has many advantages over conventional route. It has shown better patient compliance. But the major disadvantage associated with this route is that drug is unable to pass through stratum corneum, as it acts as a barrier for drug delivery. The researchers are working using various strategies such as microneedle, iontophoresis, transdermal spray, vesicles etc so that drug passes through the barrier and reaches to systemic circulation. Ultra deformable vesicles such as ethosomes, transferosomes and tranethosomes are developed. Researchers found that tranethosomes had better penetration of skin, high drug encapsulation, higher stability than ethosomes and transferosomes. Tranethosomes are elastic vesicular carrier which are developed from ethosomes (ethanol), transferosomes (edge activator) so it has advantages of both the vesicles. Tranethosomes are used in various treatments such as cancer, fungal infections, hypertension and arthritis. It is also used in delivering proteins and peptides.

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