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Formulation Development and Characterization of Drug Loaded Transethosomes for Transdermal delivery: Review Article

S.B.Gondkar[1], N.R.Patil[2*], R.B. Saudagar[3]

[1*]Department of Pharmaceutics, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.
[2]Department of Quality Assurance, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik-422213, Maharashtra, India.
[3]Department of Pharmaceutical Chemistry, R. G. Sapkal College of Pharmacy, Anjaneri, Nashik- 422213, Maharashtra, India.

Abstract : Ultradeformable vesicles (UDV) have recently become a promising tool for the development of improved and innovative dermal transdermal therapies. 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 in ability to cross intercellular channels of stratum corneum.To overcome this drawback of conventional lipidicsystems. Ethanol based vesicular carriers were developed by pharmaceutical scientist. Transethosome come under the category of ethanol based lipidic carriers. Transethosomes are composed of phospholipid, ethanol and edge activators or permeation enhancers. Ethanol based vesicular systems represent non-invasive carriers which enable the drug to reach in deeper epidermal layers or systemic circulation. Drug actives were incorporated in UDV formulations further characterized for vesicles imaging by transmission electron microscopy, mean vesicle size and; zeta potential by laser Doppler anemometry; stability and entrapment efficiency. Transethosomes may contain both advantages of Transferosomes and ethosomes. The nature methods of preparation, and evaluation parameters of transethosomes were discussed in this review along with their applications, problematic issues and future progress.
Keywords: Edge activator, Transethosomes, Transdermal drug delivery, Vesicular Permeation enhancers, In vitro study.

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 is include electrically assisted methods like iontophoresis sonophoresis, and electrophoresis etc.also the micri-invasive techniques, Vesicular systems,and also the use of permeation enhancers.The transdermal delivery is enables direct entry of bioactive molecules into the cystemic circulation, bypass of hepatic metabolism, improvement of patient compliance, and low risk to the injury of

tissue. Abioactive molecules should characterized like low molecular weight (<500Da),high pharmacological activity, high effectiveness of low doses(5-10mg/day),and high lipophilicity for the achievement of good results. Various classes of the drugs fulfill the criteria are analgesics, antianginals, contraceptives and antihypertensive drugs. nowadays the vesicular systems is mostly investigated approaches for the transdermal drug delivery. The vesiclrs are the colloidal systems in which the hydrophilic core is surrounded by amphiphilic molecules in a double layer edfasion.Vesicular systems have capability to encapsulate wide variety of drug viz.hydrophilic, lipophilic and charged hydrophilic,and amphiphilic.The effectiveness of a vesicular systems as a carrier depends on various physicochemical characteristics like surface charge,size ,thermodynamic phase, and lamellarity.The conventional liposomes show the drawback of less permeation into the deeper region of skin and they accumulate at the outer layer of stratum corneum. Transfersomes and the liposomes having the addition of edge activator like span 60, Span80, span25, tween80, and Sodium deoxycholate and sodium cholate. Transfersomes improve the skin deposition of many drugs. But they can't reach the stratum corneum deep enough. Ethosomes are the composition of the phospholipid, ethanol and water and fluidization caused by ethanol may increases intercellular space between corneocytes and enhance the skin permeation.so the transethosomes is represent the novel lipidic formulation that encompasses the advantage of both transfersomes and ethosomes.The Transethosome show the presence of high amount of ethanol with edge activator or the permeation enhancers like oleic acid.[5-12]

The following figure shows the composition of the transethosomes.

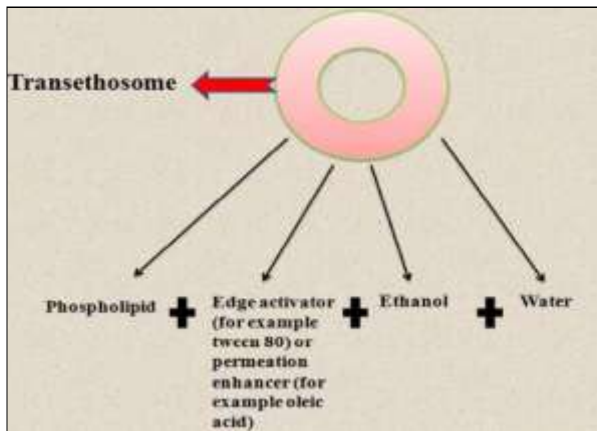

Figure 1. Composition of transethosomes.

Transethosomes have high ethanol content along with either presence of edge activator or permeation enhancer.

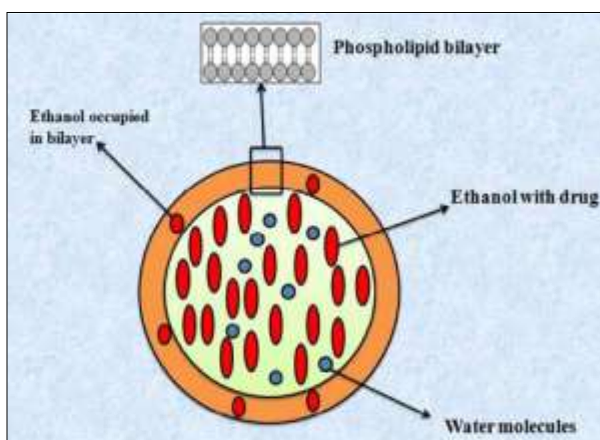

Figure 2: Schematic structure of nanoethosomal system.

The novel lipid vesicles is also known as the deformable or elastic liposomes-ultradeformable vesicles(UDV) were developed in the beginning of the 1990's.The UDV are more deformable than the conventional liposomes. They have the great ability to intact the skin and deliver the drug into the epidermis and dermislayers or even to the systemic circulation.

Currently,there are many types of UDV that have been successfully developed for both pharmaceuticals and cosmeceuticals.particularlyfothetransferosomes,ethosomes,andrecently transethosomes.(Figure2).[12-20]

Transethosomes are lipid vesicles which are combinly based or made up of transfersomes and ethosomes. It was first introduced by Song et al in 2012 where he characterized the high content of ethnol(up to 30%).Transethosomes contain the both advantages of transfersomes and ethosomes. Transethosomes have the irregular spherical shape and higher values in both vesicles elasticity and skin permeation/penetration studies. This cause due to the rearrangement of lipid bilayer in the combination of ethanol and edge activator. The fluorescent probes or dyes with the different physicochemical properties can also incorporate into the UDV for the fluorescence studies. [21-30]

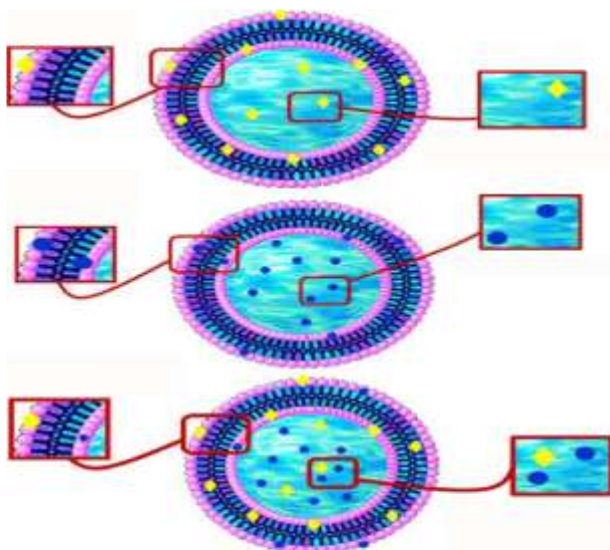

Figure 3: Schematic representation of ultradeformable vesicles.Note: (A) Transfersomes. (B) Ethosomes. (C) Transethosomes.

The penetration of the transethosomes mechanisms is described in 3 way.

1. The interaction between hydrophilic lipid and water makes the polar lipid to attract water molecules induce hydration, lipid vesicles moved to the site of higher water concentration the difference in water contents across skin stratum and epidermis develops transdermal osmotic gradients that leads to penetration of transethosomes across skin.
2. Transethosomes induce hydration that widen pores due to it there is gradual release of drug occurs that binds to targeted organ.
3. Transethosomes act as penetration enhancer which disrupt the intercellular lipids ,which results in widen of pores and increase the penetration of system through skin.[30-36]

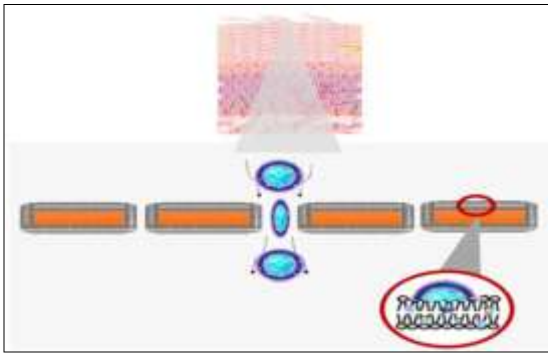

Figure 4: Schematic representation of ultradeformable vesicles permeation and penetration through the skin

Table no.1:Composition

Composition (% w/v)	Transfersomes	Ethosomes	Transethosomes
Active compound	1	1	1
SPC	10	10	10
Surfactant	Sodium cholate (NaCo): 2.3 (3.75:1, molar ratio SPC: NaCo) or Sorbitanlaurate (Span® 20): 1.8 (2:1, molar ratio SPC: Span 20)	–	Sodium cholate (NaCo): 2.3 (3.75:1, molar ratio SPC: NaCo) or Sorbitanlaurate (Span® 20): 1.8 (2:1, molar ratio SPC: Span 20)
Ethanol	–	30	30
Purified water	Qs 100	Qs 100	Qs 100

Abbreviations: Qs, quantum satis; SPC, soybean phosphatidylcholine.

Salient features of transethosomes:

1. They have high entrapment efficiency,as they are biocompatible and biodegradable in nature.
2. Encapsulated drug is protected from the degradation as due to which they release their content slowly and gradually.
3. Easy to prepare ,does not involve tedious process and also avoid the unnecessary use of pharmaceutical additives, can be used for both systemic as well as topical delivery.[37-40]
4. The drug having low molecular and high molecular weight drug can be entrapped.[41-43]
5. They are highly flexible so have higher flux rate across skin and higher rate of skin penetration as comparison to other vesicular systems.[44,45]

Advantages

- The transethosomal drug is administrated in a semisolid form.
- Enhanced drug permeation through skin for transdermal drug delivery.
- Avoidance of first pass metabolism.

Disadvantage

- Skin irritation or allergic reaction on contact dermatitis.
- Product loss during transfer from alcoholic and water media.
- Unsuccessful vesicles formation can coalesceTransethosome.

Table no 2:Comparison of transethosomes with different vesicles [1,2]

Sr.No	Method	Advantage	Disadvantage
1	Liposomes	Phospholipid vesicle, biocompatible, Biodegradable	Less skin penetration less stable
2	Proliposome	Phospholipid vesicle, more stable than liposomes	Less penetration, cause aggregation and fusion of vesicles
3	Physical methods e.g. iontophoresis	Increase penetration of intermediate size charged molecule	Only for charged drugs, transfer Efficiency is low (less than 10%)
4	Niosomes	Non-ionic surfactants vesicles	Less skin penetration easy handling But will not reach up to deeper skin layer
5	Proniosomes	Greater stability, Will convert into noisome insitu, stable	Less skin penetration easy handling But will not reach up to deeper skin layer
6	Transfersomes and Protransfersomes	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
7	Transethosomes	• Enhanced drug permeation through skin for transdermal drug delivery. • Raw material in the formulation isnontoxic in nature. • More stable. • The transethosomal drug is administrated in a • Semisolid form. • Biocompatible and Biodegradable. • Avoidance of first pass metabolism.	• Product loss during transfer from alcoholic and water media. • Skin irritation or allergic reaction on contact dermatitis • Unsuccessful vesicle formation can coalescetransethosome.

Table no 3:Ideal Composition of UDV.

Composition	Transferosome	Ethosome	Transethosome
API	Present	Present	Present
Phospholipid	Present	Present	Present
Edge activator	Present	Absent	Present
Alcohol	Absent	Present	Present
Purified water	Q.S	Q.S	Q.S

Abbrevation(API) Active Pharmaceutical Ingredients

Method of Preparation

- Cold method
- Hot method
- Thin film hydration method
- Classic mechanical dispersion method
- Classic method

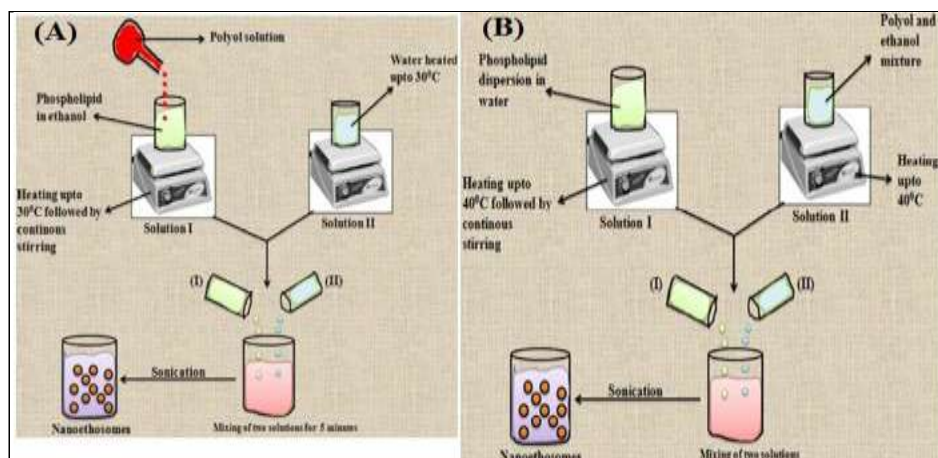

Figure 5: Preparation of transethosomes by cold and hot technique.
(A) Coldtechnique involves mixing of two solutions at a low temperature of 30 oC, and
(B) hot technique involves mixing of two solutions at a high temperature of40 oC.

Cold method:[46]

The transethosomes are prepared by using cold method, which is also used for to prepared ethosomes.Phospholipon 90G was dissolved in ethanol in a conical flask. The mixture was heated to 30°C.Sodium deoxycholate and API was dissolved in water and heated to 30°C in a separate vessel. This aqueous phase was then added to the alcoholic phase slowly in a fine stream with constant stirring(mechanical stirrer,Remi Mumbai)AT 700rpm in a closed vessel. Stirring was continued for additional 5min.The system waskept at 30°C throughout the preparation.

Thin film hydration method:

Spc (final concentration of 36 mg/ml), permeation enhancers and IM (final concentration of 0.5 mg/ml) were dissolved in 25 ml chloroform–methanol (4:1, v/v). The lipid mixture was deposited as a thin film in a round-bottom flask by rotary evaporating the chloroform–methanol under reduced pressure at 35 ± 1 C, which was applied for 1 h to ensure total removal of solvent traces. The lipid film was hydrated with 10 ml phosphate buffer solution and achieved within the eluates.

Modified hand shaking, lipid film hydration technique[47]

Drug, lecithin (PC) and edge activator were dissolved in ethanol: chloroform (1:1) mixture. Organic solvent was removed by evaporation while hand shaking above lipid transition temperature (43°C). A thin lipid film was formed inside the flaskwall with rotation. The thin film was kept overnight for complete evaporation of solvent. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature. The transfersome suspension was further hydrated up to 1 hour at 2-80°C.

Characterization of transethosomes[46,47]

A.Visualization by transmission electron microscopy (TEM)

Shape and morphology of the TELs was investigated using transmission electron microscopy. TELs were negatively stained with 2% w/v aqueous solution of phosphotungstic acid on a carbon-coated copper grid. The grid was examined under transmission electron microscope (Philips CM 200) with resolution of 2.4A° at accelerating voltage of 200 kV.

B.Determination of entrapment efficiency

Entrapment efficiency of TELs was determined by ultracentrifugation method . TELs were separated by ultracentrifugation at 15,000 rpm for 60 minutes at a temperature of 4℃. The sediment and supernatant liquid were separated, the amount of drug in the sediment was determined by rupturing the vesicles using methanol

and the amount of drug was quantified spectrophotometrically. Entrapment efficiency was determined by the following equation;

% Entrapment efficiency = Amount entrapped API x 100.

C.Determination of vesicle size and zeta potential:

The particle size and zeta potential of freshly prepared TELs was determined by Nanoparticle tracking analysis (NTA 2.3) using Nano sight NS500 with automated sample introduction, computer controlled motorized stage with CCD camera and red (638nm) laser .

D. Surface morphology study:

Different types of lipids influence the surface morphology or shape of the particles. Lipidmicro particle suspensions were deposited on metallic stubs then placed in liquid nitrogen and dried under vacuum. The freeze-dried micro particles were coated uniformly with gold. It is characterized for morphology and surface properties using a scanning electron microscope.

E.Interaction study by using DSC and FTIR:

Interaction study between the lipid and drug can be determined by using DSC. The transition temperature (Tm) of the vesicular lipid systems is determined by using the Mettler DSC 60 computerized with Mettler Toledo star software system (Mettler, Switzerland).The transition temperature was measured by using the aluminum crucibles at a heating rate 10 degree/minute within a temperature range from 20º-300ºC. Interaction study can also be done by FTIR.

F.Vesicle size and Zeta potential:

Particle size and zeta potential can be determined by dynamic light scattering (DLS) using a computerized inspection system and photon correlation spectroscopy (PCS). The size of transethosomes ranges between tens of nanometers to microns and is influenced by the composition of the formulation. Zeta potential is an important and useful indicator of particle surface charge, which can be used to predict and control the stability. In general, particles could be dispersed with proper stability when the absolute value of zeta potential was above 30mV due to the electric repulsion between particles.

G.Drug Content:

Drug content of the transethosomes can be determined using UV spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic method.

H. SurfaceTension:

Activity Measurement The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer.

I. Penetration and Permeation Studies:

Depth of penetration from transethosomes can be visualized by confocal laser scanning microscopy (CLSM).

J. Stability of Ethosome:

The ability of transethosomal formulations to retain the drug was checked by keeping the preparations at different temperatures, i.e.25±2°C (room temperature), 37±2°C and 45±2°C for different periods of time. The stability of ethosomes can also be determined quantitatively by monitoring size and morphology of the vesicles using DLS and TEM.

K. Drug Content:

The % drug content of ethosomal preparation was determined by using following formula

$$\%\text{drug content} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}}$$

Problematic issues and future progress related to ethanol based carrier systems for transdermal drug delivery.[48, 49]

Most of the bioactive molecules do not pass through stratum corneum barrier. Ethanol based Nano carriers have opened a new window to deliver various bioactive molecules trans dermally 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 e xploration 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.

Application:

1. Better permeation for anti-inflammatory activity.
2. Improved transdermal flux.
3. Increase skin penetration.
4. For transdermal immunization.
5. Increase entrapment efficiency and skin permeation.
6. Ethosomes are used in pilosabeceous targeting.
7. Transdermal Delivery of Hormones.
8. Delivery of Anti-Arthritis Drug.
9. Delivery of Antibiotic.

Conclusions:

The results obtained from this study indicate, new phospholipid carrier transethosomes which consists of high concentration of ethanol and edge activator enhances the permeation. Due to its enhanced penetration as compared to Ethosomes, hydroethanolic drug solution and plain drug solution. *In-vivo* studies showed better anti-inflammatory activity for both TELs and Els due elastic nature of the carriers as compared to the marketed formulation. Hence, the elastic formulation TELs was found to be more effective as compared to the Els as it contains both ethanol and edge activator which further enhances its transdermal permeation. The development of ethanol based vesicular carriers like transethosomes is a promising approachfor 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 passeffect, short half-life, GIT irritation, less penetration, etc. 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 Nano medicine to deliver drugs having solubility/permeability problems through transdermal route.

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