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ALCOHOLIC NANO-CARRIERS: ETHOSOME FORMULATION TECHNIQUE, CONSTITUENT AND ITS EVALUATION: A REVIEW

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ABSTRACT

There are many disadvantage of transdermal drug delivery because of its poor penetration of most of the compounds into the human skin. Many different approaches have been developed to debilitate the skin barrier. Ethosome are the main approaches which are used for increasing the skin penetration of drugs and many cosmetic chemicals. Ethosomal systems are novel lipid vesicular carriers containing a relatively high percentage of ethanol. Ethosomes is a vesicular carrier comprise of hydroalcoholic or hydro/alcoholic/glycolic phospholipid in which the concentration of alcohols or their combination is relatively high. These types of Nano-carriers are especially designed for effective delivery of medicinal substance with different physicochemical properties into deep skin layers and across the skin. There are many different preparation techniques which are used for the preparation of these types of new carriers. The prepared ethosomes were spherical and discrete in shape. The size of vesicles were found to be in the range of 3.25-5.69 µm, 0.715-1.302 µm and 5.42 µm for unsonicated ethosomes, sonicated ethosomes and liposomes respectively. For simple application and stability, Ethosomal dispersions are incorporated into patches, cream and gels. Ethosome, are non-invasive delivery carriers that enable drugs to reach deep into the skin layers or the systemic circulation made up of phospholipids, high concentration of ethanol and water. This article provides a detailed review of the ethosomal systems and categorizes them on the basis of their constituents to classical ethosomes, binary ethosomes, and transethosomes. The differences among these systems are discussed from several perspectives, including the formulation, entrapment efficiency, skin-permeation properties, size, zeta potential, and stability. This review article gives a detailed information on the effects of ethosomal system constituents, preparation methods, and their significant roles in determining the final properties of these nanocarriers. Furthermore, the novel pharmaceutical dosage forms of ethosomal gels, patches, and creams are highlighted.

KEYWORDS: Ethosome, nano-carriers, phospholipids, delivery system, zeta potential.

INTRODUCTION

Skin is the largest and most easily accessible organ of the body; it serves as a potential route of drug administration for systemic effects. The outer most layer of the skin, the stratum corneum, represents the most resistible barrier to drug penetration across the skin, which limits the transdermal bioavailability of drugs. Therefore, the special carriers are required to combat natural skin barrier to deliver drug molecules with different physicochemical properties to the systemic circulation. Ethosomes are innovative nanovesicles containing the drug in a matrix of lipids, ethanol and water. The ethosomes are soft and a highly flexible vesicle efficiently penetrates through the skin and increases the drug delivery of drug molecules. Ethosomes are elastic vesicles made up of Phospholipids containing 25-55% ethanol. Ethanol also acts as a penetration enhancer by dissolving the skin lipids. Ethosomes overcomes the disadvantages of Liposomes and proliposomes such as less stability, scalability issues, leakage of drugs, and fusion of vesicles and breaking of vesicles. Ethanol is a well-known permeation enhancer. Ethosomes is highly flexible which permits the elastic vesicles to squeeze themselves among the skin pores. Ethanol gives the net negative charge on the surface of ethosome vesicles due to which aggregation is avoided because of electrostatic repulsion. Ethosomes are much more stable than the Liposomes and proliposomes. Topically administered ethosomes increases the residence time of the drug molecule in the different layers of skin such as stratum corneum, epidermis and reduces the systemic absorption. Because of all these properties, ethosomes get easily permeated in the deeper layer of skin and circulation. Ethanol in deeper layers of skin leads to disruption of the skin which increases the lipid fluidity that allows enhanced permeation of drug molecule through the skin.

Ethosomes fuses with the skin lipids to release the drug into the deeper layers of skin.[1-4] Transdermal drug-delivery systems offer many advantages, such as avoidance of first-pass metabolism by the liver, controlled delivery of drugs, reduced dosing frequency, and improved patient compliance, as they are noninvasive and can be self-administered.[5, 6] For the benfits of intravenous drug evidence of drug infusion can be closely duplicated, without its hazards, by using the skin as the port of drug administration to provide continuous transdermal drug infusion into the systemic circulation.[7] Ethosomes have been shown to exhibit high encapsulation efficiency for a wide range of molecules including lipophilic drugs. This could be explained by multilamellarity of ethosomal vesicles as well as by the presence of ethanol in ethosomes which allows for better solubility of many drugs. Ethosomes were reported to improve *in vivo* and *in vitro* skin delivery of various drugs. Contrary to deformable liposomes, ethosomes are able to improve skin delivery of drugs both under occlusive and non-occlusive conditions.[8] Ethosomal systems differ from liposomes because they contain relatively high concentrations of ethanol, in addition to phospholipids and water.[9] The recent generations of ethosomal systems have been introduced since then by adding other compounds to the basic ethosomal formula in an attempt to enhance the vesicular characteristics and skin permeation. Therefore, till the date, there had been no clear distinction among the classical ethosomes and their newer generations. In this article we are trying to provide a detailed review of ethosomal systems and identify the different types of these vesicles based on the compounds used in their production and the impact of these compounds on ethosomal properties. This article also emphasis on the ethosome preparation methods and pharmaceutical dosage forms, and its evaluation of these promising nanocarriers for dermal/transdermal delivery.

TYPE OF ETHOSOMES SYSTEM

Classical ethosomes

Classical ethosomes are a modification of classical liposomes and are composed of phospholipids, a high concentration of ethanol up to 50% w/w, and water. Classical ethosomes were reported to be superior over classical liposomes for transdermal drug delivery because they were smaller and had negative zeta-potential and higher entrapment efficiency. Moreover, classical ethosomes showed better skin permeation and stability profiles compared to classical liposomes.[10,11] The molecular weights of drugs entrapped in classical ethosomes have ranged from 130.077 Da to 24 kDa.[12, 13]

Transethosomes

Transethosomes are the new generation of ethosomal systems and were first reported by C.K. Song in 2012.[14] Ethosomal system contains the basic components of classical ethosomes and an additional compound, such as a penetration enhancer or an edge activator (surfactant) in their formula. This novel vesicle was developed in an attempt to combine advantages of classical ethosomes and deformable liposomes (transferosomes) in one formula to produce transethosomes. There are many different types of edge activators and penetration enhancers had been investigated to produce ethosomal systems with better characteristics. Transethosomes was reported to entrap drugs with molecular weights ranging from 132.078 Da to 202–328 kDa.[15, 16]

Binary ethosomes

Binary ethosomes were introduced by Y. Zhou.[17] Mainly; it was developed by adding another type of alcohol to the classical ethosomes. The most commonly used alcohols in binary ethosomes are propylene glycol and isopropyl alcohol.[18 – 22]

PREPARATION OF ETHOSOMES

Step 1: Solution A
Soya lecithin or Cholesterol were added in ethanol under stirring and continue stirring until it dissolves completely.

Step 2: Solution B
Tramadol HCl is added in of the remaining quantity of ethanol under stirring at room temperature and stirring is continued at room temperature until it forms the clear solution.

Step 3
Add Solution A to Solution B under ultra-shear homogenization and continue ultra-shear homogenization at speed 6000 rpm for 30 min.

Step 4
Add purified water under ultra-shear homogenization drop wise in the centre of the container. The dispersion is further homogenized for 15 min at room temperature with homogenization speed 6000 rpm.

Step 5
Formulated Ethosomes forms the cloudy homogenous liquid. The Ethosomes were stored at temperature 4°C ± 0.5°C for further evaluation.[23-26]

Method of Preparation of ethosomes
Ethosomes can be prepared in a simple manner and usually does not require state of the equipments using a cold method. However, few methods like, hot method, thin-film hydration technique, and transmembrane pH gradient methods have also been reported for the preparation of ethosomes.

Cold method
This is the most commonly followed simplest method for preparing ethosomes and does not require any special equipment, and the process can be easily scaled up. Briefly, the lipid and drug will be dissolved in ethanol (or mixture of ethanol and glycols), followed by addition of sufficient amount of water as a slow stream with constant stirring for some time. Proper care should be taken to prevent the evaporation of ethanol, and the temperature during the whole preparation should be maintained at 30^0C.[4]

Hot method
This method is similar to the cold method except for the fact that the ethanolic mixture is heated to 40^0C.[27]

Thin-film hydration technique
The lipids will be dissolved using organic solvent in a round bottom flask, and the organic solvent is evaporated above the lipid transition temperature using a rotary evaporator. The thin film formed around the inner walls of the round bottom flask will be hydrated using ethanolic mixture and dispersed with a probe Sonicator to obtain a suspension of ethosomes.[28]

Mechanism of permeation across the skin barriers
The Ethosomes are widely explored for the delivery of the drugs across the skin. Fluidizing effect of ethanol on the lipid bilayer of the stratum corneum together with the softness of the ethosomal carrier gives them the capability to penetrate the perturbed subcutaneous lamellae more easily, thus promoting delivery of the actives into the deep layers of the skin and through the skin. The proposed hypothetical mechanism of action of ethosomal system for its enhanced permeation across the skin. 1st ethanol interacts and disturbs the organization of the stratum corneum lipid bilayer and enhances its lipid fluidity by reducing the melting point. Flexible ethosome vesicles can then penetrate the disturbed stratum corneum bilayer. During the process of penetration, the ethosomes fuse with the lipids present in the skin along its pathways and promote release of the drug at various points. When compared to other vesicles, occlusion slightly increases the penetration of ethosomes across the skin, which indicates that an osmotic gradient across the skin is not necessary. These data differ from that observed with elastic vesicles where permeation enhancement occurred only in non-occlusive conditions

and points toward different mechanisms of action of the carriers.[29, 30]

Effects of excipients used on ethosomal system properties

Ethanol – as penetration enhancer

Substances that reversibly reduce the barrier resistance of the stratum corneum are known as chemical penetration enhancers. Ethanol is an efficient penetration enhancer.[31] It is one of the most commonly used permeation enhancers and plays an important role in ethosomal systems by giving the vesicles unique characteristics in terms of size, zeta-potential, stability, entrapment efficacy, and enhanced skin permeability. The concentrations of ethanol in ethosomal systems have been reported to be 10% – 50%.[32] Many researchers concluded that when the concentration of ethanol is increased, the size of the ethosomes would decrease.[33–39] As a solvent, ethanol can be included in the formulation to enhance the solubility of the drug. This is particularly important for poorly soluble permeates, as they are prone to depletion in the donor vehicle. Ethanol is a relatively volatile solvent and will rapidly evaporate at skin temperature. The high ethanol concentration in ethosomes has shifted the vesicular charge from positive to negative.[40] Ethanol loss from a formulation may lead to the drug becoming supersaturated, which will influence drug flux across the membrane. Ethanol acts as a negative charge provider for the surface of ethosomes, thereby avoiding aggregation of the vesicular system due to electrostatic repulsion. Additionally, ethanol was also reported to have stabilizing effects.[41] The authors investigated the effect of ethanol on skin water content and concluded that formulations containing high levels of alcohol were capable of dehydrating the skin, which may explain the concentration dependant action of ethanol. Ethanol also has a significant effect on ethosomal system entrapment efficiency, and in general increasing ethanol concentration will increase entrapment efficiency.[42] Due to this reason, ethanol concentration should be optimized during the formulation process, as at low concentrations entrapment efficacy will be minimal, and at very high concentrations ethosomal membrane will be more permeable because phospholipids can easily be dissolved in ethanol, leading to a significant reduction in entrapment efficiency.

Cholesterol

Cholesterol is a rigid steroid molecule, and its incorporation in ethosomal systems enhances the stability and entrapment efficiency of drugs. It prevents leakage and reduces vesicular permeability and vesicular fusion. Generally, it is used at a concentration of, 3.5%.[43] But in some formulations it was used up to 70% of the total phospholipid concentration in the formulation.[44, 45] The increased rigidity (i.e. decreased elasticity) of the ethosomal vesicles upon the addition of cholesterol was also reported by other researchers.[46]

Dicetyl phosphate

Dicetyl phosphate is commonly used to prevent aggregation of the vesicles and enhance the stability of the formula. It is used at concentrations between 8% and 20% of the total phospholipid concentration in the ethosomal formulation. Maestrelli reported that all ethosomal formulations containing Dicetyl phosphate produced vesicles with sharply negative zeta-potential.[47]

Phospholipids

Phospholipids from different sources have been used in ethosomal system formulation. The selection of phospholipid type and concentration for the formulation are important factors during the development of ethosomal system because they will influence the size, entrapment efficacy, and zeta-potential, stability, and penetration properties of the vesicles. The different types of phospholipids like - Phospholipon 90H and 80H and soy phosphatidylcholine in the preparation of ethosomal systems of alfuzosin hydrochloride.[48] Highly negatively charged vesicles were produced by the incorporation of DPPG (1, 2-dipalmitoyl-*sn*-glycero-3-phosphatidylglycerol) in the ethosomal formulation.[49] Increasing phospholipid concentration will increase vesicular size slightly or moderately but will increase entrapment efficiency significantly. However, the relationship is true only until a certain concentration, whereby further increment in phospholipid concentration will have no effect on entrapment efficiency.[39]

Stearylamine

It is a positive-charge agent, and has been used in ethosomal formulations in two studies. First study involved an ethosomal system consisting of phosphatidylcholine: cholesterol: Stearylamine at a molar ratio of 2:1:1 and loaded with Mycophenolic The lower permeation of the positively charged ethosomes was due to the shielding effect of Stearylamine and the competition of the positively charged Stearylamine with positively charged vancomycin hydrochloride. Stearylamine penetrates the skin easily because it has smaller molecular weight (296.5 Da) compared to vancomycin hydrochloride (1,458.7 Da). Stearylamine will cross the skin more easily than vancomycin hydrochloride.[50]

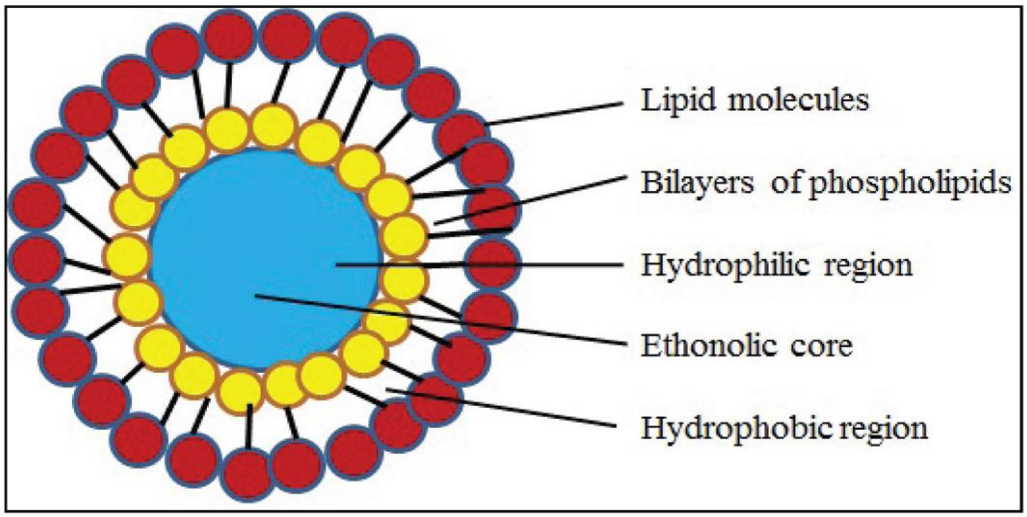

Figure 1: Structure of ethosome.

Ethosomes Purification

The purification of ethosomes was determined by dialysis membrane method. Cellulose acetate membrane (molecular weight cutoff 50,000; Himedia) was kept in saline solution for 2 hours 30 minutes before dialysis to ensure complete wetting of the membrane. 01 milliliter of the drug-loaded vesicles was placed in a dialysis bag, which was then transferred into 500 mL of phosphate buffered saline, pH 6.8. The receiver medium was stirred with a magnetic stirrer at 500 rpm. Ten-milliliter samples were withdrawn after 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hours and replaced with an equal volume of fresh dissolution medium.[51]

Ethosomes Characterization

After the preparation of ethosomes, it is highly necessary to characterize them on the basis of particle size, morphology, zeta potential, entrapment efficiency, physical state of the entrapped drug, and permeation studies across the skin. Particle size can be measured by dynamic light scattering or photon correlation spectroscopy using Malvern particle size analyzer. The dynamic light scattering measures the average hydrodynamic diameter of particles by measuring the changes in the speckled pattern produced by the scattered light of the particles in Brownian motion. For the identification of the presence of organic functional groups in the prepared ethosomal formulations, FTIR study was performed. IR Spectra of all prepared formulations were obtained with FTIR Spectrophotometer, Perkin Elmer Spectrum. The scanning range was 4000-400cm-1 at a scan period of 1 minute.[52] The surface charge of the ethosomes can also be measured using Malvern particle size analyzer using a suitable probe (zeta dip cell). Zeta potential provides information about the stability of the ethosomes during storage. The morphology of the ethosomes can be visualized by atomic force microscope and transmission electron microscope. By using transmission electron microscope, 1D, 2D, and 3D imaging, measuring, modeling, and manipulating matter can be accomplished. It is based on the absorption of electron beam as it passes through ultrathin (<100 nm) samples. The AFM utilizes piezoelectric ceramics to move a specimen in nanoscale increments in the X, Y, and Z directions. Basic principle of atomic force microscope depends on the measurement of the interactive force between a tip and the sample surface using special probes made by an elastic cantilever with a sharp tip on the end. For the amount of drug entrapped in the ethosomes can be measured by separating the ethosomes from the free drug. By centrifuging the sample at high speed, heavier ethosomes form sediment at the bottom by leaving out the free drug in the supernatant. Estimating of the free drug would give an idea on the amount of drug entrapped in the ethosomes. Using differential scanning Calorimetry, the transition temperature of the vesicular lipid systems and the physical state of the entrapped drug can be identified. Using excised skin, the rate and the ability of the ethosomes to permeate the skin can be measured. Additionally, the mechanism by which the ethosomes permeate the skin can also be visualized by confocal laser scanning microscope. Surface morphology of optimized ethosome was observed by Scanning Electron Microscope. Optimized ethosome was mounted on a glass stub, air-dried and coated with gold using a sputter coater and finally, visualized under a JEOL JSM - 6490LA scanning electron microscope at an accelerating voltage of 15 kV.[53]

Evaluation of ethosomes

Vesicles shape: Shape and Size of ethosomes were measured by the digital motic microscope. Ethosomes are dispersed in Mineral oil. Ethosomes dispersion is placed on the glass slide and focused under 100x object lens. The image was snapped by digital motic microscope using three different fields.[54-56]

Zeta potential: Zeta potential of ethosomes was measured using laser dropper anemometry using Zetasizer. Ethosomes were dispersed in purified water. The dispersion of ethosomes was placed in the

electrophoretic cell where the potential of 150 mV was established. The analysis is performed in triplicate.

Vesicles size: Formulated ethosomes were analyzed by Malvern Sizer for vesicle size. Ethosomes were dispersed in purified water and the dispersion was placed in a clear disposable zeta cell for 90 seconds. The reading was taken in triplicate.

Optical microscopy: The Ethosomes are diluted with Liquid paraffin oil and mounted on glass slides. The dispersion is fixed with cover slip on the glass slide. The Microscopic examination is conducted using Motic Digital Microscope under 40X object lens. The 500 particles were calculated from different field and mean and a median is calculated.

Drug entrapment efficacy: Entrapment efficacy of ethosomes was performed by Ultra- centrifugation method. The indirect method is used to determine entrapment efficiency by measuring the unentrapped drug. The ethosomes have subjected the dispersion for ultracentrifugation at 24000 rpm for 60 min. The clear supernatant layer is removed. The supernatant layer and sediment were analyzed for Tramadol HCl content by UV Spectrophotometer at 271 nm. Each sample was prepared in duplicate.[57, 58]

$$\text{Entrapment Efficiency (\%)} = \frac{\text{Amount of drug in sediment} \times 100}{\text{Total amount of drug in supernatant and sediment}}$$

In-vitro drug release for ethosomes: Ethosomes were spread on egg membrane equivalent amount 100 mg of drug uniformly. This egg membrane is mounted on the donor compartment of Franz diffusion cell. The receptor compartment is filled with 24 ml of pH 7.4 Phosphate buffer. 1 ml Samples are withdrawn at specific time points 15 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 8 hr, 10 hr and 12 hr. To maintain the sink condition at each time point replaces the withdrawn sample amount with fresh buffer solution. The study is carried out for 12 h.[59-61]

Application of Ethosomes as a Carrier System

Various studies employing ethosomal formulation have shown better skin permeability of drugs. The uses of ethosomes as carrier system for transdermal/topical drug delivery are summarized below.[62-64]

Pilosebaceous targeting

Hair follicles and sebaceous glands are increasingly being recognized as potentially significant elements in the percutaneous drug delivery. Interest in pilosebaceous units has been directed towards their use as depots for localized therapy, particularly for the treatment of follicle-related disorders such as acne or alopecia. Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery. With the purpose of pilosebaceous targeting, Maiden et al. prepared and evaluated Minoxidil ethosomal formulation. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness. Conventional topical formulation has very poor skin permeation and retention properties. It was found that the quantity of Minoxidil accumulated into nude mice skin after application of its ethosomal formulation was 2.0, 7.0 and 5.0 fold higher as compared to ethanolic phospholipids dispersion, hydroethanolic solution and ethanolic solution of drug each containing 0.5% of the drug. These results showed the possibility of using ethosomes for pilosebaceous targeting of Minoxidil to achieve its better clinical efficacy.

Transdermal delivery of hormones

Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several dose dependent side effects. In addition, along with these side effects oral hormonal preparations relying highly on patient compliance. The risk of failure of treatment is known to increase with each pill missed. Touitou *et al.* compared the skin permeation potential of testosterone ethosomes (Testosome) across rabbit pinna skin with marketed transdermal patch of testosterone. They observed nearly 30-times higher skin permeation of testosterone from ethosomal formulation as compared to that marketed formulation. The amount of drug deposited was significantly. The AUC and Cmax of testosterone significantly improved after application of Testosome as compared to Testoderm. Hence, 4.06, 19.15 and 19.33, 0.06) higher in case of ethosomal formulation (132.78) in vitro and in vivo studies demonstrated improved skin permeation and bioavailability of testosterone from ethosomal formulation. This group in their further study designs the testosterone nonpatch formulation to reduce the area of application. They have found that with ethosomal testosterone formulation area of application required to produce the effective plasma concentration was ten times less than required by commercially gel formulation.[65-66]

Topical delivery of DNA

Many environmental pathogens attempt to enter the body through the skin. Skin therefore, has evolved into an excellent protective barrier, which is also immunologically active and able to express the gene. On the basis of above facts another important application of ethosomes is to use them for topical delivery of DNA molecules to express genes in skin cells. Touitou et al. in their study encapsulated the GFP-CMVdriven transfecting construct into ethosomal formulation. They applied this formulation to the dorsal skin of 5-week male CD- 1 nude mice for 48 hr. After 48 hr, treated skin was removed and penetration of green fluorescent protein (GFP) formulation was observed by CLSM. It was observed that topically applied ethosomes-GFP-

CMV-driven transfecting construct enabled efficient delivery and expression of genes in skin cells. It was suggested that ethosomes could be used as carriers for gene therapy applications that require transient expression of genes. These results also showed the possibility of using ethosomes for effective transdermal immunization. Gupta et al. recently reported immunization potential using transfersomal formulation. Hence, better skin permeation ability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents.[67-68]

Delivery of anti-arthritis drug

Topical delivery of anti-arthritis drug is a better option for its site-specific delivery and overcomes the problem associated with conventional oral therapy. Cannabidol is a recently developed drug candidate for treating rheumatoid arthritis. Its oral administration is associated with a number of problems like low bioavailability, first pass metabolism and GIT degradation. To overcome the above mention problem prepared Cannabidol -ethosomal formulation for transdermal delivery. Results of the skin deposition study showed significant accumulation of Cannabidol in skin and underlying muscles after application of Cannabidol -ethosomal formulation to the abdomen of ICR mice Plasma concentration study showed that steady state level was reached in 24 hours and maintained through 72 hours. Significantly increased in biological anti-inflammatory activity of Cannabidol -ethosomal formulation was observed when tested by carrageenan induced rat paw edema model. Finally, it was concluded that encapsulation of Cannabidol in ethosomes significantly increased its skin permeation, accumulation and hence it's biological activity.[69]

The reverse-phase evaporation method

This is the least used method and specially designed to produce large unilamellar vesicles. The organic phase is prepared by dissolving the phospholipid in diethyl ether and then mixing it with the aqueous phase at a ratio of 3:1 v/v in an ultrasonic bath at 0°C for 5 minutes to form a water-in-oil emulsion. The organic solvent is removed under reduced pressure to produce a gel, which turns into a colloidal dispersion upon vigorous mechanical agitation.[47]

Ethosomal gels

Ethosomal gels are characterized for their pH, viscosity, spreadability, and extrudability. The most commonly used gel-forming agents for incorporating ethosomal systems are Carbopol andhydroxypropyl methylcellulose with all their related grades. These polymers have been shown to be compatible with ethosomal systems, providing the required viscosity and bioadhesive properties. Several researchers have studied the skin-permeation and disposition properties of drugs from ethosomal gels in comparison to the traditional or marketed gels or creams. Puri and Jain compared the in vitro skin-permeation properties of 5-fluorouracil from ethosomal gel and marketed cream using Franz diffusion cell and albino rat skin and found that the transdermal flux of 5-fluorouracil from ethosomal gels was 4.9-fold higher than the marketed cream. Moreover, the skin disposition of the drug from the ethosomal gel was 9.4-fold higher than the marketed cream.[70] Some authors have reported the same superior properties of ethosomal gels for different drugs/agents over traditional gels.[71] Interestingly, it was found that the drug-release rate from the ethosomal suspension was faster than from ethosomal gel, due to the high viscosity of the gel.[72–74]

Preparation of gel:

Step 1: Ethosomes equivalent to 1% of Tramadol HCl is calculated.

Step 2: Dissolve Disodium EDTA under stirring in purified water. Carbopol 980 is added under stirring and allows hydrating under stirring for 60 min.

Step 3: Ethosomes are dispersed in the hydrated Carbopol 980 slurry under stirring and stirring continued for 30 min.

Step 4: pH of the formulation is adjusted to 4.30 to 4.70, a thick gel is formed.[75, 76]

Ethosomal creams

There have only been two studies reporting the formulation of ethosomal creams. Both of these involved the incorporation of *Curcuma longa* extract-loaded ethosomal systems in a cream base as a photo protective and antiwrinkle agent. In both studies, *C. longa* extract-loaded ethosomal creams were applied to human volunteers and showed promising results as either a photo protective or an antiwrinkle agent.[77,78] Based on all the aforementioned studies, the incorporation of ethosomal systems in suitable vehicles such as gels, patches, and creams improves skin permeation of the drug/agent from the ethosomal systems. Among the vehicles discussed, gels are the most suitable vehicle for

the incorporation of ethosomal systems, while ethosomal creams may be preferred for cosmetic preparations.

Mechanisms of ethosomal system skin permeation

Ethanol and phospholipids are reported to act synergistically to enhance the skin permeation of drugs in ethosomal formulations. Ethanol fluidizes the lipid bilayer of the ethosomal vesicles and the stratum corneum simultaneously, changing the arrangement and decreasing the density of skin lipids. Therefore, the highly malleable and soft vesicles of an ethosomal system will penetrate the altered structure of the stratum corneum and create a pathway through the skin. The release of the therapeutic agent occurs by the fusion of these vesicles into cell membranes in the deeper layers of the skin.[79–83]

It is suggested that transethosomes have superior skin-permeation properties over classical ethosomes. This is because transethosomes contain both ethanol and the edge activator or the penetration enhancer, which both act together to increase vesicular malleability and skin-lipid perturbation.[84, 85]

CONCLUSION

It should be easily concluded that ethosomes have better skin permeation than liposomes. Ethosomes had more advantages when compared to transdermal and dermal delivery system. It has been almost two decades since the invention of ethosomes, and during this period these nanocarriers have proven their unique ability to deliver therapeutic agents of different physicochemical properties through the skin for local and systemic use. The incorporation of ethosomal systems in suitable vehicles such as gels, and creams represents an important step to get better skin-permeation and therapeutic results. However, more studies are required to enhance the stability of the ethosomal system. The results of the in vivo studies and clinical trials are reflecting the potential of ethosomal systems in dermal and transdermal delivery of therapeutic and cosmetic agents. They are the noninvasive drug delivery carriers that enable drug to reach the deep skin layers finally delivering in the systemic circulation. Its delivers large molecules such as peptides, protein molecules. Ethosomes are characterized by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. The main limiting factors of transdermal drug delivery system i.e. epidermal barrier can be overcome through ethosomes to significant extent. Ethosomes have been proved to the interesting delivery systems for pharmaceutical and cosmetic products; topically applied ethosomes can increase the residence time of drugs or cosmetic chemicals in the stratum corneum and epidermis and reduce the systemic absorption of drugs or cosmetic chemicals, these properties allow them to penetrate easily into the deeper layers of the skin and circulation. Ethosomal carrier opens new challenges and opportunities for the development of novel improved therapies. Further, research in this area will allow better control over drug release in vivo and long term safety data, allowing the therapy more effective.

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