ETHOSOMES: A NOVEL APPROCH FOR VESICULAR DRUG DELIVERY SYSTEM

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Abstract - Ethosomal systems are newer lipid vesicular carriers that have been around for 20 years, but over that period they have grown significantly Ethosomes are phospholipid-based elastic nanovesicles containing a high content of ethanol (20-45%). Ethosomal systems are much more efficient in delivering substances to the skin in the terms of quantity and depth, than either conventional liposomes or hydroalcoholic solutions. Ethosomes are able to encapsulate and distribute extremely lipophilic molecules through the skin, as well as cationic drugs, due to their unique structure. Ethosomal systems are novel vesicular lipid carriers, which have a relatively high ethanol contentThis article gives a review of ethosomes including their compositions, types, mechanism of drug delivery, stability, and safety behavior. This article also provides a detailed overview of drug delivery applications of ethosomes in various diseases.

Index Terms - Ethosomes, Novel drug delivery, characterization and applications of vesicular formulations, ethosomes, nanotechnology, preparation

I. INTRODUCTION

Lipoidal vesicles with high concentration of ethanol called as Ethosomes. Ethosomes are also known as ethanolic liposomes. Ethanol imparts unique characteristics to ethosomes, including ,small vesicular size, which ranges from tens of nanometres to microns depending on its composition ,high deformability , fluidity and stability Therefore, several studies suggest that ethosomes are more effective in improving the extension and efficiency the significance of ethosomes as efficient nanocarriers towards skin delivery of active ingredients, based on their characteristics and composition, as well as to understand the mechanism of permeation of these nanocarriers through the skinand tissue[4,5,6]

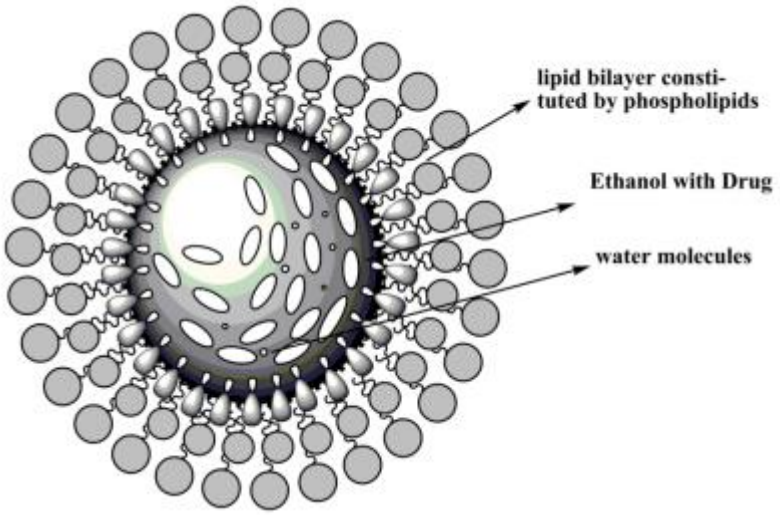

Fig.1 Structure of Ethosomes

SALIENT FEATURES:

Transfersomes are prepared from phospholipids which are biocompatible, biodegradable and nontoxic. Transfersomes are able to provide sustained drug delivery and can act as a carrier for hydrophilic and hydrophobic drugs, as well as low and high molecular weight drugs. They are also reported to be especially successful in cases, where drug is rapidly cleared off by blood capillaries. Drug release from these vesicles is concentration dependent. At higher drug concentration, systemic delivery of drug is generally obtained, whereas, at intermediate concentration dermal delivery of drug is obtained. Thus, based on drug concentration, they are capable of providing controlled delivery of drugs to systemic circulation as well to skin. [2,3]

II. TYPES OF ETHOSOMES

Ethosomal system types illustrates the three types of ethosomal systems, classified on the basis of their compositions.

1. Classical Ethosomes

Classical ethosomes are a modification of classical liposomes and are composed of phospholipids, a high concentration of ethanol up to 45% 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 ζ-potential and higher entrapment efficiency. Moreover, classical ethosomes showed better skin permeation and stability profiles compared to classical liposomes.6–8 The molecular weights of drugs entrapped in classical ethosomes have ranged from 130.077 Da to 24 kDa.

2. Binary Ethosomes

Binary Ethosomes were introduced by Zhou et al. Basically, they were developed by adding another type of alcohol to the classical ethosomes. The most commonly used alcohols inbinary ethosomes are propylene glycol (PG) and isopropyl alcohol (IPA).

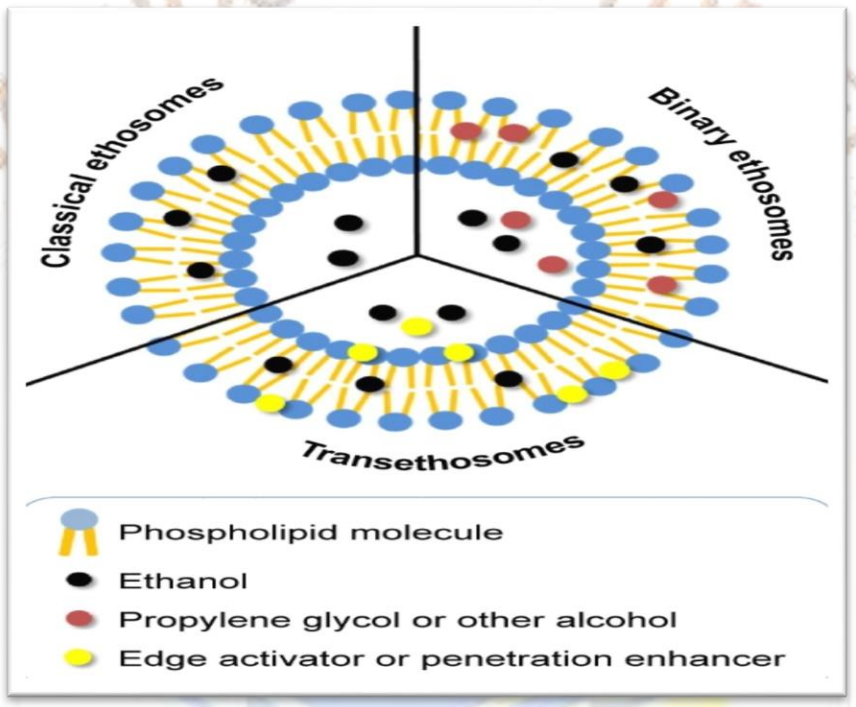

Fig.2 Types of Ethosomes

3. Transethosomes

Transethosomes are the new generation of ethosomal systems and were first reported by Song et al in 2012. This 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. These novel vesicles were developed in an attempt to combine the advantages of classical ethosomes and deformable liposomes (transfersomes) in one formula to produce transethosomes. Many researchers have reported superior properties of transethosomes over classical ethosomes.17–30 Different types of edge activators and penetration enhancers have been investigated to produce ethosomal systems with better characteristics. Transethosomes were reported to entrap drugs with molecular weights ranging from 130.077 Da to 200–325 kDa.18,21 Table 1 shows the comparison of classical ethosome, binary ethosome, and transethosome properties in their initial suspension form. [8,13]

III. Mechanism of drug delivery:

The drugs get permeated through the skin into the systemic circulation. The mechanism of drug delivery of ethosomes through permeation in not clearly understood. There may be two reasons for permeation.

i. Effect of ethanol
ii. Effect of Ethosomes

The feature of ethanol got a breakthrough in 1996 where it gets a platform of formulation consisting phospholipid, double distilled water(DDW) and propylene glycols (PG) etc, and the visage ofethosomes emerged (Touitou, 1996). On application of the ethosomal system to the skin a number of associated process takes place involving the stratum corneum and pilosebaceous pathways. [16,17,18]

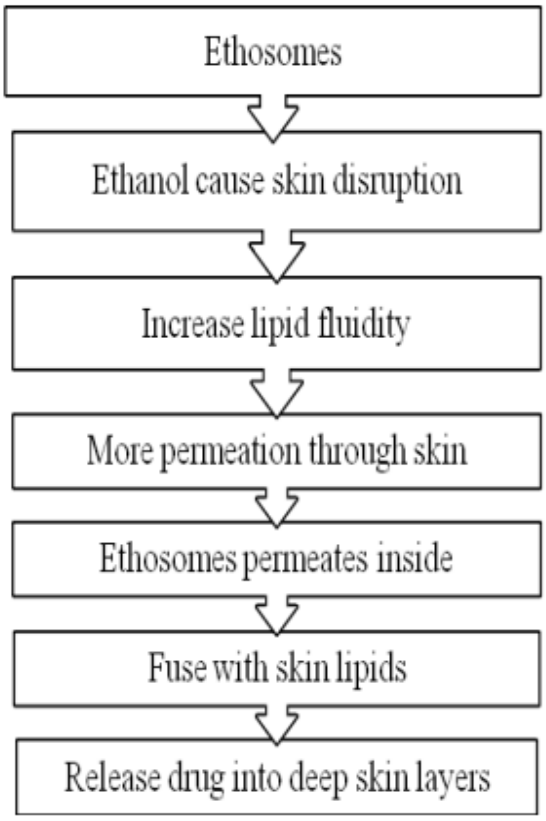

Fig.3 Mechanism of drug delivery

Advantages of Ethosomal drug delivery:

1. Increasing efficacy and therapeutic index.
2. Reduction in toxicity of the encapsulated agent.
3. Improved permeation: Ethosomes are efficient method of drug delivery that improve permeation of drug through skin. In contrast
4. Delivery of large molecules (peptides, protein molecules) is possible.
5. It contains nontoxic raw material in formulation.
6. High patient compliance the ethosomal drug is administered in semisolid form (gel or cream) hence producing high patient compliance.
7. Ethosomal system is passive, non-invasive and is available for immediate commercialization.
8. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
9. Peptides and protein particles of tremendous and distinctive social affair of prescriptions can be passed on using ethosomes
10. Parts used in ethosomes are ensured and embraced for pharmaceutical likewise, remedial use. [1,10,14]

Disadvantages of Ethosomal Drug Delivery:

1. Ethosomal administration is not a means to achieve rapid bolus type drug input, rather it usually designed to offer slow, sustained drug delivery.
2. Adequate solubility of the drug in both lipophilic and aqueous environments to reach dermal microcirculation and gain access to the systemic circulation.
3. Product may loss during transfer from organic media to aqueous media.

4. High manufacturing cost.

5. May not be economical. [4, 11]

IV. METHOD OF PREPARATION OF ETHOSOMES

Method of preparation There are two methods which can be used for formulation and preparation of ethosomes. Both of these are very simple and convenient and do not involve any sophisticated instrument or complicated process.

1. Hot Method: In this method phospholipid is dispersed in water by heating in a water bath at 400 °C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400 °C. Once both mixtures reach 400 °C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/

hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method. [14]

2. Cold method: In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 300 °C in a water bath. The water heated to 300 °C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be de-creased to desire extendusing sonication or extrusion method. Finally, the formulation is stored under refrigeration [7]

3. Filming-rehydration and Ultrasonic method: Curcumin ethosomes was prepared by filming-rehydration and ultrasonic method (Chen Jin et al., 2010). The prepared ethosome consist of 1~3% (w/v) lecithin, 30 to 45% ethanol (v/v), curcumin (0.1%) and water. For preparation of ethosome, an amount of lecithin and that of curcumin were dissolved in a glass bottle and mixed well with a magnetic stirrer. The glass bottle was connected to an injector and sealed; thereafter ethanol was added without vaporization. The mixture was poured into a round bottom flask and a thin film was prepared using roto-evaporator. The above mentioned procedures was repeated. Double distilled water (100 ml) was added to rehydrate the film to obtain the methyl nicotinate ethosomes. Then the ethosomes were homogenized for 5 min using a sonde-type ultrasonic instrument. Subsequently, the ethosomes were filtered using a 0.22 μm disposable filter. All the procedures in this test were carried out under gaseous nitrogen at room temperature. The quality fractions of curcumin and methyl nicotinate were 0.1 and 0.2%, respectively. Curcumin was not added in the aforementioned preparation process to produce empty ethosome suspension. [12]

V. CHARACTERIZATION OF ETHOSOMAL FORMULATION

1. Optical microscope observation:
The ethosomal dispersion is spread on the glass slide with the help of a glass rod. Prepare the multilamellar vesicles were detected by examining the ethosomal suspension using an optical microscope with the magnification power of 100 X.

2. Visualization:
Visualizing ethosomes can be done using instrument transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Visualizing an ethosomal formulation by electron microscopy reveals exhibited vesicular structure 300-400 nm in diameter.

3. Transition temperature:
Scanning calorimetric can measure the transition temperature of the vesicular lipid systems.

4. Drug content:
UV spectrophotometer can determine drug substance or content of the ethosomes. It can also be quantified by a modified high-performance liquid chromatographic method.

5. Vesicle Size and Zeta potential particle size:
The ethosomes can be detected by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). The Zeta potential of the ethosome suspension can be measured by the Zeta meter. [20]

VI. EVALUATION OF ETHOSOMES

1. Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy:
It involves application of vesicle suspension (0.2 mL) to filter membrane having a pore size of 50 nm and placing it in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with phosphate buffer saline solution, (having pH 6.5). The filters were removed after 1 hour and were prepared for SEM studies by fixation at 4°C in Karnovsky's fixative overnight followed by dehydration with graded ethanol solutions (30%, 50%, 70%, 90%, 95%, and 100% v/v in water). Finally, filters were coated with gold and examined in SEM.

2. Statistical Analysis:
Statistical significance of all the data generated was tested by employing ANOVA followed by studentized range test. A confidence limit of $P < .05$ was fixed for interpretation of the results using the software PRISM.

3. Stability Study:
Stability of the vesicles was determined by storing the vesicles at 4°C ± 0.5°C. Vesicle size, zeta potential, and entrapment efficiency of the vesicles was measured after 180 days using the method described earlier.

4. Drug Uptake Studies:

The uptake of drug into MT-2 cells (1×106 cells/mL) was performed in 24-well plates (Corning Inc) in which 100 µL RPMI medium was added. Cells were incubated with 100 µL of the drug solution in PBS (pH 7.4), ethosomal formulation, or marketed formulation, and then drug uptake was determined by analyzing the drug content by HPLC assay.

5. HPLC Assay:

The amount of drug permeated in the receptor compartment during in vitro skin permeation experiments and in MT-2 cell was determined by HPLC assay using methanol: distilled-water: acetonitrile (70:20:10 vol/vol). mixture as mobile phase delivered at 1 mL/min by LC 10-AT vp pump (Shimadzu, Kyoto, Japan). A twenty -microliter injection was eluted in C-18 column (4.6×150 mm, Luna, 54, Shimadzu) at room temperature. The column eluent was monitored at 271 nm using SPDM10A vp diode array UV detector. The coefficient of variance (CV) for standard curve ranged from 1.0% to 2.3%, and the squared correlation coefficient was 0.9968.

6. Statistical Analysis:

Statistical significance of all the data generated was tested by employing ANOVA followed by studentized range test. A confidence limit of $P < .05$ was fixed for interpretation of the results prism (GraphPad, Version 2.01, and San Diego, CA). [1,9]

VII. APPLICATIONS OF ETHOSOMES

Ethosomes, the high ethanol derived vesicles are capable of penetrating deeper layers of the skin and thus tend to be vesicles of choice for transdermal drug delivery via the skin of hydrophilic and impermeable drugs.

1. Hormone delivery:

Oral hormone delivery is related to numerous issues, such as high first-pass metabolism, poor oral bioavailability and many dose-dependent side effects. In addition, oral hormonal preparations which depend heavily on patient compliance with these side effects. The risk of treatment failure is known to rise with every missed pill. Touitou et al. Revealed ability of ethosomes in hormonal delivery by performing a comparative analysis of transdermal delivery of testosterone loaded ethosomes (Testosome), as compared to transdermal testosterone patch (Testoderm patch, Alza) through rabbit pinna skin, which showed approximately 30-times higher skin permeation of testosterone from ethosomal formulation. For ethosomal formulation, the volume of drug deposited was substantially (p50.05) higher (130.76 ± 18.14 and 18.32 ± 4.05 mg at the end of 7 h for Testosome and Testoderm, respectively. The area under the curve (AUC) and Cmax of testosterone significantly improved after the application of Testosome as compared to Testoderm. Thus, both in vitro and in vivo studies have shown increased skin permeation and testosterone bioavailability from ethosomal formulation.

2. Transcellular delivery

Ethosomes have been shown to be an effective penetration enhancer and carrier device for the transcellular delivery of various therapeutic agents in active clinical trials. In contrast, almost no fluorescence was observed when integrated in a hydroethanolic solution or classic liposomes. After 3 min of incubation, the intracellular existence of each of the three tested probes was evident.

3. Pilosebaceous targeting

The percutaneous drug delivery of hair follicles and sebaceous glands is increasingly recognized as potentially significant elements. The interest in pilosebaceous units was directed to their use as depots for localized therapy, particularly for the treatment of follicle-related disorders such as acne or alopecia. In addition, extensive attention has also been paid to using the follicles as transportation shunts for systemic drug delivery.

4. Delivery of anti-parkinsonism agent

Dayan and Touitou prepared ethosomal formulations of the psychoactive drug trihexyphenidyl hydrochloride (THP) and contrasted their delivery from traditional liposomal formulations. THP is an antagonist of M1 muscarinic receptors and used to treat Parkinson's disease. The transdermal flux value of THP from ethosomes via the nude mouse skin was 87, 51 and 4.5 times higher than that of liposome, phosphate buffer, and hydroethanol solution, respectively. After application of ethosomes, the amount of THP remaining in the skin at the end of 18 hr was substantially higher than after application of liposome or hydroethanolic (control) solution. Such findings revealed a greater potential for skin permeation of ethosomal-THP formulation and its use to help treat Parkinson disease.

5. Topical delivery of DNA

A lot of environmental pathogens are trying to get into the body through the skin and skin has developed into an outstanding defensive barrier that is both immunologically active and capable of expressing the gene. The important use of ethosomes on the basis of the above facts is to use them for the topical delivery of DNA molecules to express genes in skin cells. It has been proposed that ethosomes may be used as carriers for applications for gene therapy that require transient gene expression. The findings also suggested the ability to use ethosomes for successful transdermal immunization. Therefore improved ethosomal skin permeation capacity opens the possibility of using these dosage types to deliver immunizing agents.

6. Delivery of anti-arthritis drug

Topical delivery of anti-arthritis medication is a better alternative for site-specific delivery and overcomes traditional oral therapy-related problems. Cannabidol (CBD) is a drug candidate recently discovered to treat rheumatoid arthritis. His oral administration is associated with a variety of issues such as low bioavailability, first pass metabolism, and degradation of GIT. Significantly increased in CBD-ethosomal formulation biological antiinflammatory activity was observed when examined by the

carrageenan mediated rat paw edema model. Thus, it was concluded that encapsulation of CBD in ethosomes greatly increased its permeation of the skin, its accumulation and thus its biological activities.

7. Delivery of Antibiotics

Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of these agents. Conventional oral therapy causes several allergic reactions along with several side effects. Conventional external preparations possess low permeability to deep skin layers and subdermal tissues. [18] Ethosomes can circumvent this problem by delivering sufficient quantity of antibiotic into deeper layers of skin. Ethosomes penetrate rapidly through the epidermis and bring appreciable amount of drugs into the deeper layer of skin and suppress infection at their root. With this purpose in mind Godin and Touitou prepared bacitracin and erythromycin loaded ethosomal formulation for dermal and intracellular delivery. The results of this study showed that the ethosomal formulation of antibiotic could be highly efficient and would overcome the problems associated with conventional therapy.

8. Bronchial asthma, chronic bronchitis, and emphysema:

Ehab R. Bendas, et al., compared the transdermal delivery of salbutamol sulfate (SS), from ethosomes and classic liposomes containing various cholesterol and dicetylphosphate concentrations. The vesicle size was significantly decreased by decreasing cholesterol concentration and increasing concentrations of dicetylphosphate and ethanol. The entrapment efficiency percentage was significantly increased by increasing concentrations of ethanol, cholesterol and dicetylphosphate. [13,15,19]

SAFETY OF ETHOSOMES:

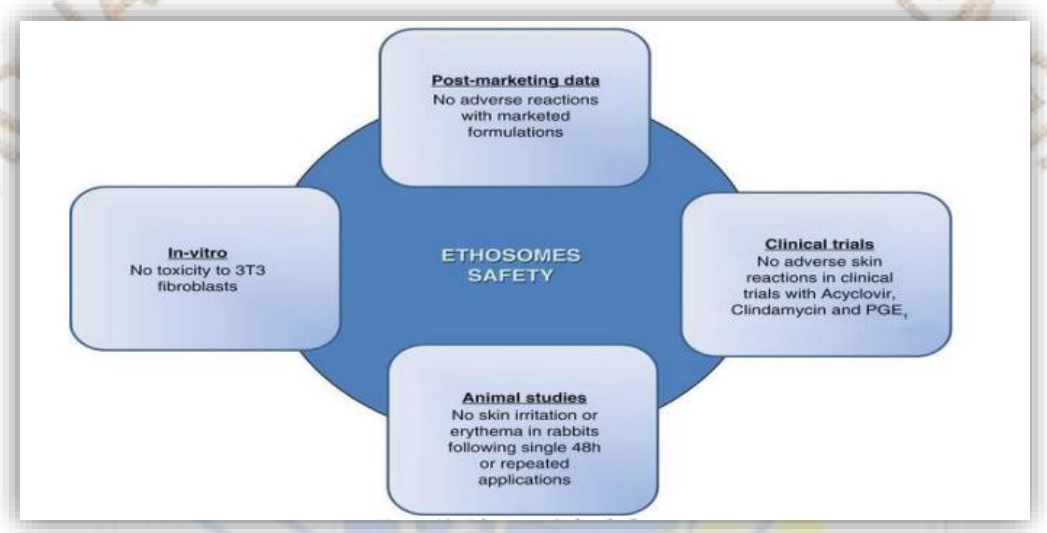

Fig.4 Safety of Ethosomes

VIII. CONCLUSION:

A novel drug delivery method serves to improve therapeutic value by lowering toxicity and requiring less frequent administration to overcome noncompliance boosting the bioavailability, and so on. To include herbal medicines into innovative drug delivery systems, a great deal of research is being done on them. Ethosomes have been tested to encapsulate hydrophilic drugs, cationic drugs, proteins and peptides. Various ethosomal preparations are currently available in the market. However, more studies are required to enhance stability of ethosomes.

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