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A REVIEW ON ETHOSOMES: NOVEL DRUG DELIVERY SYSTEM

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ABSTRACT

Ethosomes are non-invasive delivery carriers that enable drugs to reach the deep skin layers and the systemic circulation. Ethosomes are composed of phospholipid, alcohol, polyglycol and water. Ethanol increases the penetration rate of the skin and delivers the drug into the deeper layers of skin. Ethosomes are widely used instead of liposomes due to its improved drug delivery, penetration rate. Enhanced delivery of bioactive molecules through the skin and cellular membranes by means of an ethosomal carrier opens numerous challenges and opportunities for the research and future development of novel improved therapies.

INTRODUCTION

Ethosomes are novel carrier system used for delivery of drugs having low penetration through the biological membrane mainly skin. Ethosomes are the slight modification of well established drug carrier liposome. Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water. The size range of ethosomes may vary from tens of nanometers to microns (μ). Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux in comparison to conventional liposomes. Although, the exact mechanism for better permeation into deeper skin layers from ethosomes is still not clear. The synergistic effects of combination of phospholipids and high concentration of ethanol in vesicular formulations have been suggested to be responsible for deeper distribution and penetration in the skin lipid bilayers. Ethosomes are mainly used for the delivery of drugs through transdermal route. The transdermal delivery is one of the most important routes of drug administration. The main factor which limits the application of transdermal route for drug delivery is the permeation of drugs through the skin. Human skin has selective permeability for drugs. Lipophilic drugs can pass through the skin but the drugs which are hydrophilic in nature can't pass through. Water soluble drugs either show very less or no permeation. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. Ethosomes can entrap drug molecule with various physicochemical characteristics i.e. of hydrophilic, lipophilic, or amphiphilic. The size range of ethosomes may vary from tens of nanometers to

microns (μ). Ethosome formulations provide sustained delivery of drugs where ethosomes act as reservoir system for continues delivery of drugs. Visualization by transmission electron microscopy showed that ethosomes could be unilamellar or multilamellar through to the core. The size of ethosome vesicles varies from tens of nanometre to a few microns depending on method of preparation, composition and application techniques like sonication. Contrary to Transfersomes, ethosomes improves skin delivery of drugs both under occlusive and non-occlusive condition.[1,8]

ADVANTAGES OF ETHOSOMAL DRUG DELIVERY

1. Delivery of large molecules (peptides, protein molecules) is possible.
2. It contains non-toxic raw material in formulation.
3. Enhanced permeation of drug through skin for transdermal drug delivery.
4. Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
5. High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
6. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods
7. The Ethosomal system is passive, non-invasive and is available for immediate commercialization[3,5]

ETHOSOMES COMPOSITION: Ethosomes are vesicular carrier comprise of hydro alcoholic or hydro/alcoholic/glycolic phosphor lipid in which the concentration of alcohols or their combination is relatively high. Typically, Ethosomes may contain phospholipids with various chemical structures like phosphatidyl choline (PC), hydrogenated PC, phosphatidic acid (PA), phosphatidyl serine (PS), phosphatidyl ethanolamine (PE), phosphatidyl glycerol (PPG), phosphatidyl inositol (PI), hydrogenated PC, alcohol (ethanol or isopropyl alcohol), water and propylene glycol (or other glycols). Such a composition enables delivery of high concentration of active ingredients through skin. Drug delivery can be modulated by altering alcohol: water or alcohol-polyol: water ratio. Some preferred phospholipids are soya phospholipids such as Phospholipon 90 (PL-90). It is usually employed in a range of 0.5-10% w/w. Cholesterol at concentrations ranging between 0.1 1% can also be added to the preparation. Examples of alcohols, which can be used, include ethanol and isopropyl alcohol. Among glycols, propylene glycol and Transcutol are generally used. In addition, non-ionic surfactants (PEG-alkyl ethers) can be combined with the phospholipids in these preparations. Cationic lipids like cocoamide, POE alkyl amines, dodecylamine, cetrimide etc. can be added too. The concentration of alcohol in the final product may range from 20 to 50%. The concentration of the non-aqueous phase (alcohol and glycol combination) may range between 22 to 70%[3,6,7]

METHODS OF PREPARATIONS OF ETHOSOMES

Ethosomal formulation may be prepared by hot or cold method as described below. Both the methods are convenient, do not require any sophisticated equipment and are easy to scale up at industrial level. 1.**Cold Method:** In this method Phospholipids, 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 30ºC in a water bath. The water heated to

30ºC 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 decreased to the desire extent using probe sonication or extrusion method. Finally, the formulation is stored under refrigeration.

2. Hot Method: In this method Phospholipid is dispersed in water by heating in a water bath at 40ºC until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 40ºC. Once both mixtures reach 40ºC, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic or hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method[4,7,8]

MECHANISM OF PENETRATION

The Main Advantage Of Ethosomes Over Liposomes Is The Increased Permeation Of The Drug. The Mechanism Of The Drug Absorption From Ethosomes Is Not Clear. The Drug Absorption Probably Occurs In Following Two Phases:

1. Ethanol Effect
2. Ethosomes Effect

1. Ethanol Effect: Ethanol acts as a penetration enhancer through the skin. The mechanism of its penetration enhancing effect is well known. Ethanol penetrates into intercellular lipids and increases the fluidity of cell membrane lipids and decrease the density of lipid multilayer of cell membrane.

2. Ethosome Effect: Increased cell membrane lipid fluidity caused by the ethanol of Ethosomes results increased skin permeability. So the Ethosomes permeates very easily inside the deep skin layers, where it got fused with skin lipids and releases the drugs into deep layer of skin.[2,7,8]

METHOD OF CHARACTERIZATION OF ETHOSOMAL FORMULATION

1. Vesicle shape: Visualization of ethosomes can be done using transmission electron microscopy (TEM) and by scanning electron microscopy (SEM). Visualization by electron microscopy reveals an ethosomal formulation exhibited vesicular structure 300-400 nm in diameter. The vesicles seem to be malleable as evident by their imperfect round shape.

2. 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).

3. Drug entrapment: The entrapment efficiency of ethosomes can be measured by the ultracentrifugation technique.

4. Transition Temperature: The transition temperature of the vesicular lipid systems can be determined by using differential scanning calorimetry.

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

6.Surface tension measurement: The surface tension activity of drug in aqueous solution can be measured by the ring method in a Du Nouy ring tensiometer

3. Stability studies: The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM.

4. Skin permeation studies: The ability of the ethosomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy (CLSM)[7,8]

EVALUATION TESTS

1. Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy: Vesicle suspension (0.2 mL) was applied to filter membrane having a pore size of 50 nm and placed in diffusion cells. The upper side of the filter was exposed to the air, whereas the lower side was in contact with PBS (phosphate buffer saline solution), (pH 6.5). The filters were removed after 1 hour and 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% vol/vol in water). Finally, filters were coated with gold and examined in SEM.

2. Vesicle-Skin Interaction Study by Fluorescence Microscopy: Fluorescence microscopy was carried according to the protocol used for TEM and SEM study. Paraffin blocks are used, were made, 5- m thick sections were cut using microtome and examined under a fluorescence micro Cytotoxicity Assay MT-2 cells (T-lymphoid cell lines) were propagated in Dulbecco's modified Eagle medium containing 10% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mmol/L Lglutamine at 37°C under a 5% CO2 atmosphere. Cytotoxicity was expressed as the cytotoxic dose 50 (CD50) that induced a 50% reduction of absorbance at 540 nm.

3. Vesicle-Skin Interaction Study by TEM and SEM: From animals ultra thin sections were cut, collected on formvar-coated grids and examined under transmission electron microscope. For SEM analysis, the sections of skin after dehydration were mounted on stubs using an adhesive tape and were coated with gold palladium alloy using a fine coat ion sputter coater. The sections were examined under scanning electron microscope.

4. 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. 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.

5. Drug Uptake Studies: The uptake of drug into MT-2 cells (1×106 cells/mL) was performed in 24-well plates in which 100 L RPMI medium was added. Cells were incubated with 100 L 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.

6. Skin Permeation Studies: The hair of test animals (rats) were carefully trimmed short with a scalpel. The excised skin was placed on aluminium foil, and the dermal side of the skin was gently teased off for any adhering fat and/or subcutaneous tissue. The effective permeation area of the diffusion cell and receptor cell volume was 1.0 cm2 and 10 mL, respectively. The temperature was maintained at 32°C ± 1°C. The receptor compartment contained PBS (10 mL of pH 6.5). Excised skin was mounted between the donor and the receptor compartment. Ethosomal formulation (1.0 mL) was applied to the epidermal surface of skin. Samples (0.5 mL) were withdrawn through the sampling port of the diffusion cell at 1-, 2-, 4-, 8-, 12-, 16-, 20-, and 24-hour time intervals and analyzed by high performance liquid chromatography (HPLC) assay.

7. 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.[3,9]

THERAPEUTICS APPLICATION OF ETHOSOMES: Ethosomes can be used for many purposes in drug delivery. Ethosomes are mainly used as replacement of liposomes. Mainly the transdermal route of drug delivery is preferred. Ethosomes can be used for the transdermal delivery of hydrophilic and impermeable drugs through the skin. Various drugs have been used with ethosomal carrier.

1. **Pilosebaceous targeting:** Hair follicles and sebaceous glands are increasingly being recognized as potentially significant elements in the percutaneous drug delivery. Furthermore, considerable attention has also been focused on exploiting the follicles as transport shunts for systemic drug delivery. Minoxidil is a lipid-soluble drug used topically on the scalp for the treatment of baldness by pilosebacious delivery. Interest in pilosebaceous units has been directed towardstheir use as depots for localized therapy, particularly for the treatment of follicle-related disorders such as acne or alopecia.2.

2. Transcellular Delivery: Better cellular uptake of anti-HIV drug zidovudine and lamivudine in MT-2 cell line from ethosomes as compared to the marketed formulation suggested ethosomes to be an attractive clinical alternative for anti-HIV therapy.

3.Delivery of problematic drug molecules: Oral delivery of large biogenic molecules such as peptides or proteins and insulin is difficult because they are completely degraded in the GIT tract hence transdermal delivery is a better alternative. But conventional transdermal formulation of biogenic molecules such as peptides or protein and insulin has poor permeation. Formulating these above molecules into ethosomes significantly increase permeation and therapeutic efficacy

4. In the treatment herpetic infection : 5% acyclovir ethosomal preparation compared herpetic infections.

5. Transdermal Delivery of Hormones: Oral administration of hormones is associated with problems like high first pass metabolism, low oral bioavailability and several doses dependent side effects. The risk of failure of treatment is known to increase with each pill missed.

6. Delivery of Anti-Arthritis Drug: Topical delivery of anti-arthritis drug is a better option for its site-specific delivery andovercomes the problem associated with conventional oral therapy.

7. Delivery of Antibiotics: Topical delivery of antibiotics is a better choice for increasing the therapeutic efficacy of this 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 .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.Cosmaceutical Applications of Ethosomes: The advantage of applying ethosomes in cosmaceuticals is not only to increase the stability of the cosmetic chemicals and decrease skin irritation from

the irritating cosmetic chemicals, but also for transdermal permeation enhancement, especially in the elastic forms. However, the compositions and sizes of the vesicles are the main factors to be considered to obtain these advantages of the elastic vesicles for cosmaceuticals applications.

9.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-CMV driven transfecting construct into ethosomal formulation. They applied this formulation to the dorsal skin of 5-week male CD1 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 361 transfersomal formulation. Hence, better skin permeation ability of ethosomes opens the possibility of using these dosage forms for delivery of immunizing agents.[9]

CONCLUSION

Ethosomes are more advantages when compared to transdermal and dermal delivery. They are the noninvasive drug delivery carriers that enable drugs to reach the deep skin layers finally delivering to the systemic circulation. It provides the delivery of large molecules such as peptides, protein molecules. Ethosomes are distinguished by simplicity in their preparation, safety and efficacy and can be tailored for enhanced skin permeation of active drugs. The main limiting factor of transdermal drug delivery system i.e. epidermal barrier can be overcome by ethosomes to remarkable extent. 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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