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Review Article of Ethosomes

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ABSTRACT

Ethosomes are phospholipid nanovesicles used for dermal and transdermal delivery of molecules. Ethosomes were developed by Touitou et al.,1997, as additional novel lipid carriers composed of ethanol, phospholipids, and water. They are reported to improve the skin delivery of various drugs. Ethanol is an efficient permeation enhancer that is believed to act by affecting the intercellular region of the stratum corneum.

The domain" Ethosomes " comprises with 4 modules with different matters. Module one deals with introduction, GMP and GLP requirements. The second module illustrated with preformulation, identification and characterization methods, drug-excipient compatibility study, criteria for excipient selection, formulation optimization techniques and formulation. The third module describes the evaluation and stability studies. The fourth module includes SOP of equipments, packaging and labelling.

KEY WORD: Ethosomes

INTRODUCTION

- Ethosomes are ethanolic liposomes. They were developed by Touitou in 1997.
- Ethosomes are non-invasive delivery carrier that enable drugs to reach the deep skin layers or systemic circulation.
- Size – 30nm to few microns.
- Ethosomes are the slight modification of well established drug carrier liposome.
- Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water.
- Ethosomes are soft vesicles made of phospholipids and ethanol (in higher quantity) and water.

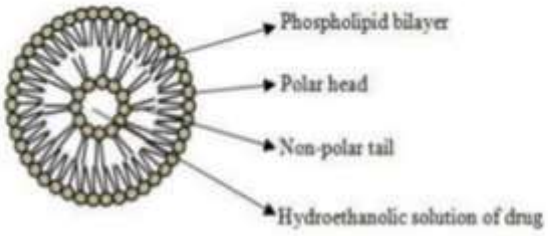

Fig.1 Structure of Ethosome

TYPES OF ETHOSOMAL SYSTEMS

Classical ethosomes

Classical ethosomes are composed of phospholipids, water, and high concentration of ethanol (40%). Because of small size, negative zeta potential and higher entrapment efficiency classical ethosomes were superior over classical liposomes. Drugs having molecular weight ranging from

130.077 Da to 24 k Da can be entrapped in classical ethosomes. Classical ethosomes also shows better skin permeation and stability profiles than classical liposomes.

Binary ethosomes

Binary ethosomes can be prepared by adding another type of alcohol to the classical ethosomes. propylene glycol (PG) and isopropyl alcohol (IPA) are the most commonly used alcohols in binary ethosomes.

Transethosomes

Transethosomes are the new form of ethosomal systems. In their formula it contain basic components from classical ethosomes and a penetration enhancer or an edge activator (surfactant). These novel vesicles were developed to combine the advantages of classical ethosomes and transfersomes in one formula to produce transethosomes.

ADVANTAGES

- Enhanced permeation of drug through skin for transdermal drug delivery.
- Delivery of large molecules (peptides, protein molecules) is possible.
- It contains nontoxic raw material in formulation. 4) High patient compliance the ethosomal drug is administered in semisolid form (gel or cream) hence producing high patient compliance.
- Ethosomal system is passive, non-invasive and is available for immediate commercialization.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields.
- Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods.
- Ethosomes are enhanced permeation of drug through skin for transdermal and dermal delivery.
- Ethosomes are platform for the delivery of large and diverse group of drugs (peptides, protein molecules)
- Ethosome components are approved for pharmaceutical and cosmetic use.
- Low risk profile -Technology has no large-scale drug development risk since toxicological profiles of the ethosomal components are well documented in the scientific literature.
- High patient compliance- The ethosomal drug is administrated in semisolid form (gel or cream), producing high patient compliance by is high. In contrast, iontophoresis and phonophoresis are relatively complicated to use which will affect patient compliance.
- High market attractiveness for products with proprietary technology. Relatively simple to manufacture with no complicated technical investments required for production of Ethosomes.

DISADVANTAGES

- Poor yield.
- If shell locking is ineffective then the coalescence of ethosomes may occur and fall apart on transfer into water.
- Loss of product during transfer from organic to water media.

PREFORMULATION STUDIES IDENTIFICATION AND CHARACTERIZATION METHODS

FTIR Analysis

Fourier Transform Infrared Spectroscopy (FT-IR) was performed to detect the active functional groups in the extract by Infrared spectrophotometer. Before obtaining the IR spectrum, the dried samples milled with potassium bromide and 300 kg/cm2 pressure was applied to the mixture to form a pallet. The KBr disks were used to develop the spectrum over a range of 4,000–400 cm-1

SEM Analysis

The surface morphology of the ethosomes was observed through Scanning Electron Microscopy (SEM). Prior to analysis, the ethosomal samples were mounted onto double-sided tape that had previously been secured on copper stubs and coated with platinum, then analyzed at different magnifications.

Entrapment Efficiency

The entrapment efficiency (EE) of ethosomes was calculated by the ultracentrifuge method. Briefly, ethosomes were kept overnight at 4°C and centrifuged in an ultracentrifuge at 12,000 rpm for 30 min. Then the supernatant was collected, diluted with water and drug concentration was determined at 290 nm in both vortex and non-vortex samples. Finally, the EE was calculated by the following equation.

EE (%)= Amount of drug in sediment x 100
Total amount of drug added

Size analysis of vesicular systems

The mean size of ethosomal colloidal suspension was analyzed by dynamic light scattering technique with a Zetasizer 3000 HSA. The sample was placed in quartz cuvette and size measurements were carried out at a scattering angle of 90°. All observations were recorded in triplicate for each formulation.

Vesicle shape

Shape and morphology of the ethosome vesicles were investigated using transmission electron microscopy. Formulation diluted with water was adsorbed onto a grid with carbon-coated formvar film that was attached to a metal specimen grid. Excess sample was blotted off and the grid was covered with a small drop of staining solution (2% w/v uranyl acetate). It was left on the grid for few minutes and excess solution was drained off. The grid was allowed to dry thoroughly in air and sample was examined in the transmission electron microscope.

Vesicle size and Zeta potential

Particle size and Zeta potential of the ethosomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy (PCS). Size and size distribution were determined by dynamic light scattering (DLS) using a computerized inspection system. Surface morphology was determined by TEM, a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aq. solution of phosphotungustic acid. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron.

In vitro drug permeation study

The in vitro permeation study was carried out by using Franz diffusion cell with egg membrane. The study was performed with phosphate buffer saline (pH 7.4). The formulation was placed on the upper side of skin in donor compartment. The temperature was maintained at 37±2º. Samples were withdrawn after every hour from the receptor media through the sampling tube and at the same time, fresh media was added to receptor to make sink condition. Withdrawn samples were analyzed for drug constant using UV/Vis spectrophotometer.

Stability studies

Formulations were stored at 4±2°, 8° and at room temperature. Percent drug entrapment was determined at different time intervals.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.

DRUG – EXCIPIENT COMPATIBILITY STUDIES

IR spectroscopy was used to investigate interactions between different components in the formulation. One part of the sample and three parts of potassium bromide were taken in a mortar and triturated well. A small amount of triturated sample was taken into a pellet maker and was compressed at 10 kg/cm2 using hydraulic press (Bruker). The pellet was kept on to the sample holder and scanned from 400 to 4000 cm-1 in IR spectrophotometer. The spectrum obtained was compared with original spectra of drug and optimized formulation.

CRITERIA FOR EXCIPIENT SELECTION

1. Lipid Component:

- Phospholipids: Choosing phospholipids with high biocompatibility (like phosphatidylcholine, phosphatidylethanolamine) to form the vesicular structure of ethosomes.
- Fluidity and Chain Length: Selecting lipids with appropriate chain length and fluidity to achieve the desired membrane fluidity and permeability.

2. Surfactants:

- Non-Ionic Surfactants: Choosing non-ionic surfactants (such as Tween, Span) to stabilize vesicles and improve their colloidal stability.
- Solubilizing Agents: Surfactants that enhance solubility and dispersion of hydrophobic drugs within the lipid bilayers.

3. Hydration Medium:

- Aqueous Phase: Selection of suitable aqueous phases (saline, buffer) to maintain stability and osmotic balance of the vesicles.

4. Compatibility:

- Drug Compatibility: Ensuring compatibility between the drug and chosen excipients to maintain drug stability, bioavailability, and efficacy.
- Excipient Compatibility: Compatibility between all excipients to avoid chemical interactions leading to instability or reduced efficacy.

5.Biocompatibility and Safety:

- Non-Toxicity: Choosing excipients that are safe and non-toxic for topical or systemic administration.

- Biodegradability: Using excipients that degrade or metabolize within the body to prevent accumulation or toxicity.

6. Vesicle Stability:

- Colloidal Stability: Excipients that enhance the stability of ethosomes against aggregation, fusion, or leakage of encapsulated drugs.
- Osmotic Stability: Ingredients that maintain osmotic balance within the vesicles to prevent swelling or shrinkage.

7. Enhanced Drug Delivery:

- Permeation Enhancers: Including excipients that can improve the penetration of drugs through biological membranes.
- Targeting Ligands: Incorporating ligands for specific targeting or enhanced cellular uptake if required.

8. Physical Properties:

- Vesicle Size Control: Excipients influencing the vesicle size distribution for the desired application (e.g., smaller vesicles for improved skin penetration).
- Zeta Potential Control: Controlling the surface charge of ethosomes for stability and interaction with biological membranes.

9. Cost and Manufacturing:

- Cost-Effectiveness: Considering the cost of excipients in formulation development without compromising quality.
- Ease of Manufacturing: Excipients that allow straightforward and scalable production processes.

FORMULATION OPTIMIZATION TECHNIQUES

Optimization is selecting the most suitable element from available influence decisions in any resources considering all the factors which in experiment. Designing quality formulation is obtained by the use of various techniques of optimization Quality by Design enhances the assurance of safe and effective drugs to consumer and promise to improve manufacturing quality performance and also product free of t as in the label to the contamination and gives the desired benefit to consumer. Design of experiment is systemic planning and performing studies that change the experimental variables to determine their effect on a given response. Optimization techniques and Experimental cally to examine various problems that occur design are used speci during the research. If the experiments in the production are carried out randomly then results obtained will be random, so we need to plan the experimental process such that relevant information is obtained. In pharmaceutical industry optimization techniques used for the drug delivery systems are designed accordingly which include,

Defining the objective

Planning the experiment

Factors which influence the is screened

Selecting the experimental designs

Formulation and evaluation of drug delivery system as instructed by experimental design

Search for the optimum by using computer aided modeling

Design of experiments methodology should be validated

Scale up and the obtained steps by this entire process is implemented in production of the desired pharmaceutical drug delivery system.

EXPERIMENTAL DESIGN

It is a statistical design that advises a set of combination of variables. Depending on the factors, levels, Interactions and order of the model various experimental designs are chosen. Includes

- Factorial design
- Fractional factorial design
- Full factorial design
- Plackett-burman design
- Central composite design (ccd)
- Box-behnken design
- Taguchi design
- Mixture design
- Star design
- Box design
- Uniform shell design (doehlert uniform shell design)
- Simplex lattice design
- D-optimal design
- Sequential optimization design
- Extreme vertices design
- Evolutionary method

FORMULATION OF ETHOSOMES

CHEMICAL	**EXAMPLES**	**USE**
Phospholipids	Soya phosphatidyl choline Egg phosphatidyl choline	Vesicles forming component
Polyglycol	Propylene glycol Transcutol	As a skin penetration enhancer
Alcohol	Ethanol Isopropyl alcohol	For providing the softness for vesicle membrane
Cholesterol	Cholesterol	For providing the stability to vesicle membrane

Fig.2. Formulation Table

EVALUATION OF FORMULATION

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. **Skin Permeation Studies:**

The hair of test animals (rats) were carefully trimmed short (<2 mm) with a pair of scissors, and the abdominal skin was separated from the underlying connective tissue 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 receptor compartment. Ethosomal formulation (1.0ml) was applied to the epidermal surface of the skin. Samples(0.5ml) were withdrawn to through the sampling port of the diffusion cell at 1-, 2-, 4-, 8-, 12-, 16-, 20,- & 24 hrs time intervals & analysed by high performance liquid chromatography (HPLC) assay.

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. **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.

5. **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-µm 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 L-glutamine 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.

6. **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.

7. **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 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.

8. **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.

STABILITY STUDIES

Stability testing is a procedure performed for all the pharmaceutical products at various stages of the product development. Depending upon the aim, steps followed, the stability testing procedures have been categorized into four types and they are:

1. Real-time stability testing

2. Accelerated stability testing

3. Retained sample stability testing

4. Cyclic temperature stress testing.

Real-time stability testing

Real-time stability testing is normally performed for a long duration of time to allow significant degradation of the product under the storage conditions recommended. The period of time for the test of the product depends on the stability of the product which clearly tells that the product is not degraded or decomposed for a long time.

Accelerated stability testing

This type of stability testing is done at higher temperatures and that decomposition the product is determined. The information is used to predict the shelf life or used to compare the relative stability of alternative formulations. The accelerated stability studies are easily predicted by the Arrhenius equation,

$K=Ae^{-Ea/RT}$

Where, K= Specific rate constant

A= Frequency factor or Arrhenius factor

Ea= Energy of activation

R= Real gas constant 4.184 j/mol. k

T= Absolute temperature

In this method the drugs are stored at different temperatures such as 40°C, 60°C, 70°C, 80°C, 100°C etc.

Retained sample stability testing

These studies are to be done at room temperature and at refrigerator temperatures. In this type of testing, the stability is done by selecting one batch for a year. If the number of samples exceeds more than 50 then they are divided into two batches. The samples stability studies help to predict the shelf life. The maximum shelf life of every product predicted could be 5 years which is conventional to the test samples at 3, 6. 9, 12, 18, 24, 36, 48 and 60 months. This method of testing is also known as constant interval method.

Cyclic temperature stress testing

This method is not so much used to the sampling of the products. In this method, cyclic temperature stress tests are designed knowledge of the product so as to mimic likely conditions in the market place storage. In this testing the sampling is considered to be conducted by a cycle of 24 hours which is known as the rhythm of the earth is 24 hours.

PACKAGING AND LABELLING OF ETHOSOMES

Packaging and labeling of ethosomes involve careful considerations to ensure their stability, safety, and compliance with regulatory standards.

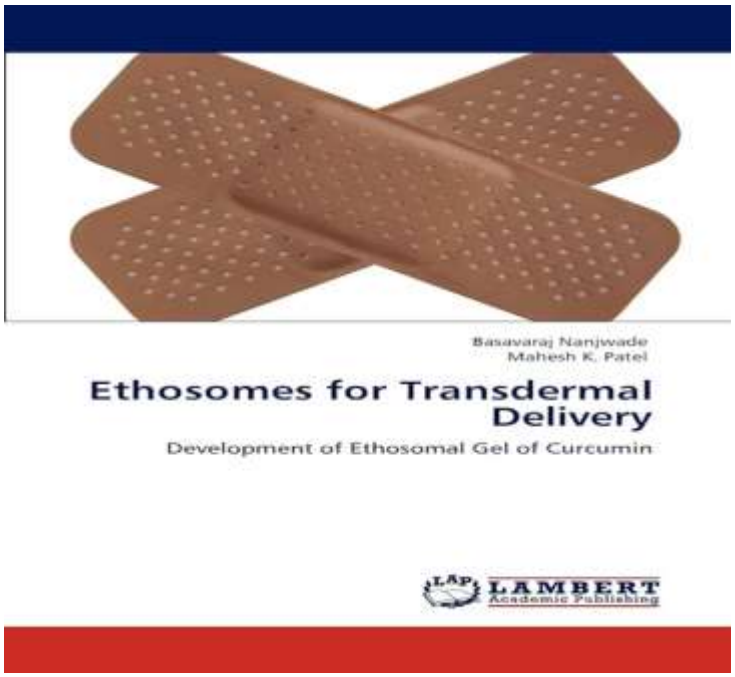

Fig.4 Packaging of Ethosomes

Packaging:

1. Container Selection: Ethosomes are typically stored in vials, tubes, or other airtight containers made of materials that don't interact with or degrade the formulation. These containers should be inert, ensuring the stability of ethosomes over time.

2. Protection from External Factors: Packaging must shield ethosomes from light, moisture, and temperature fluctuations, which can degrade the formulation. Opaque or light-protective materials are used to prevent light-induced degradation. Labels often include storage instructions specifying temperature ranges and protection from light.

3. Sealing: The containers are sealed tightly to prevent leakage and maintain the integrity of the formulation. Proper sealing also helps in preserving the sterility and efficacy of ethosomes.

4. Batch Information: Each package is assigned a batch number, enabling traceability for quality control purposes. This batch number allows manufacturers to track and recall specific products if necessary.

Labeling:

1. Product Information: Labels include essential details such as the product name, manufacturer information, and batch number for identification purposes.

2. Composition: Ethosome formulations may have various compositions. Labels should specify the key components, including phospholipids, ethanol, water, and any additional excipients.

3. Instructions for Use: Clear and concise instructions for application are provided, including recommended dosage, frequency of application, and any specific usage guidelines.

4. Storage Conditions: Instructions regarding the storage conditions necessary to maintain the stability of ethosomes. This includes recommended temperature ranges and precautions against exposure to light or moisture.

5. Expiry Date: The expiration date indicates the period until which the product is expected to remain stable and effective when stored under recommended conditions.

6. Safety Information and Warnings: Labels often include precautionary statements, contraindications, and potential adverse reactions. This information is crucial for user safety and compliance.

7. Regulatory Compliance: Labels must comply with local regulatory requirements and standards. This ensures that the product meets safety and quality standards set by regulatory authorities.

8. Barcode and Serialization: Barcodes may be included for inventory tracking purposes, while serialization enables unique identification of individual packages.

CONCLUSION

Ethosomes are non-invasive delivery carrier that enable drugs to reach the deep skin layers or systemic circulation. Here we discussed about GMP, GLP requirements, preformulation studies, identification and characterization methods, drug-excipient compatibility studies, criteria for excipient selection, formulation optimization techniques and formulation of ethosomes, also discussed about evaluation of ethosomes which includes Filter Membrane-Vesicle Interaction Study by Scanning Electron Microscopy, Skin Permeation Studies, standard operating procedures, stability studies and finally packaging and labeling requirements.

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