

PREPARATION AND CHARACTERIZATION OF NABUMETONE ETHOSOMAL GEL

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Abstract: Background: The present study aimed to develop an Ethosomal formulation for topical delivery of Nabumetone to enhance the water solubility as well as the permeability of the drug. The ethosomes were prepared by cold method, ethanol is used as a penetration enhancer. F1 to F12 different formulations of ethosomes are prepared and characterized for size and shape analysis, entrapment efficacy. Carbopol 934 is used as a gelling agent. The prepared formulation was evaluated for spreadability, in vitro diffusion study, in vivo skin permeation study. Nabumetone is NSAID belongs to bio-pharmaceutics classification system (BCS) class II drug, widely used in the treatment of osteoarthritis and rheumatoid arthritis. The most frequently reported adverse reactions for the drug involve a disturbance in the gastrointestinal tract, diarrhea, dyspepsia, and abdominal pain.

Keywords: Nabumetone, Ethosomes, topical delivery, Phospholipid, Anti-inflammatory activity.

Introduction:

Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, vaginal, rectal and skin as a topical route. Skin is the most accessible organ on the human body for topical administration. Drugs are administered topically at the site of application for systemic effect or/and local action.

Dermatological products that are applied to the skin include diverse formulations and ranges in consistency from liquid to powder, but most popular products among our semisolid preparations within the major group of semisolid preparations, the use of transparent gels has broadened both in pharmaceutical preparation and cosmetics.

Gels are a relatively newer class of dosage forms created by entrapment of large amounts of aqueous (or) hydro-alcoholic liquid in a network of colloidal solid particles. They have a higher aqueous compartment that permits the greater dissolution of drugs and also permit easy migration of drug through a vehicle that is essentially a liquid compared to ointment or cream base.

Gels are easy to apply and have high patient acceptability.

To overcome the skin's outermost layer (stratum corneum) barrier, various mechanisms have been investigated such as the use of chemical enhancers or physical enhancers.

Liposomes, Niosomes, Transferosomes, and Ethosomal vesicular drug delivery has the potential of overcoming the skin barrier and have been proved to enhance the permeability of the drug across the stratum corneum barrier¹

Introduction to Ethosomes

Ethosomes are commonly used for delivery of medicament via transdermal route, these are 3rd generation of elastic lipid carriers, developed by Touitou. Ethosomes are malleable vesicles that act with ethanol effect and lipid penetration to a deeper layer of skin. The size of ethosomes may range from tens of nanometers to microns (μ) (Patel, 2007). These are modified forms of liposomes that are high in content of ethanol. Ethanol may also provide vesicles with soft flexible characteristics, which allows them to move easily and deeper penetration of the skin.²

Ethosomes are composed mainly of phospholipids (phosphatidylcholine, phosphatidylserine, phosphatidic acid), and a high concentration of ethanol and water, the non-aqueous phase ranges from 22 to 70%.

The main advantage of ethosomes over liposomes is the increased permeation of drug. The drug absorption probably occurs through the ethanol effect, ethosome effect^{3,4}

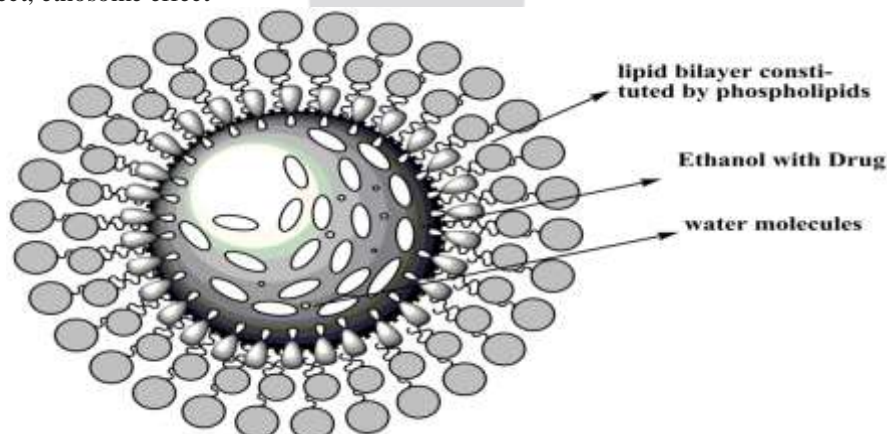


Figure 1: structure of Ethosome³

Advantage of ethosomal drug delivery system

- ✓ Ethosomes can deliver large molecules like proteins, and peptides
- ✓ Enhanced permeation of drug through skin for transdermal administration
- ✓ Simple method for the drug delivery in comparison to other techniques like Iontophoresis, Phonophoresis and other complicated methods
- ✓ The ethosomal system is passive, non-invasive and available for immediate commercialization⁵

Disadvantages of ethosomal drug delivery system: although ethosomal system has several advantages it also posses few disadvantages such as,

- ✓ The molecular size of the drug should be such that the molecule should be easily absorbed percutaneously.
- ✓ May not be economical
- ✓ Adhesive may not adhere well to all types of skin.

Mechanism of Drug penetration of ethosomal formulation: The main advantage of ethosomes over liposomes is the increased permeation of the drug. The drug absorption probably occurs in the phases:

1. Ethanol
2. Ethosome effect⁶

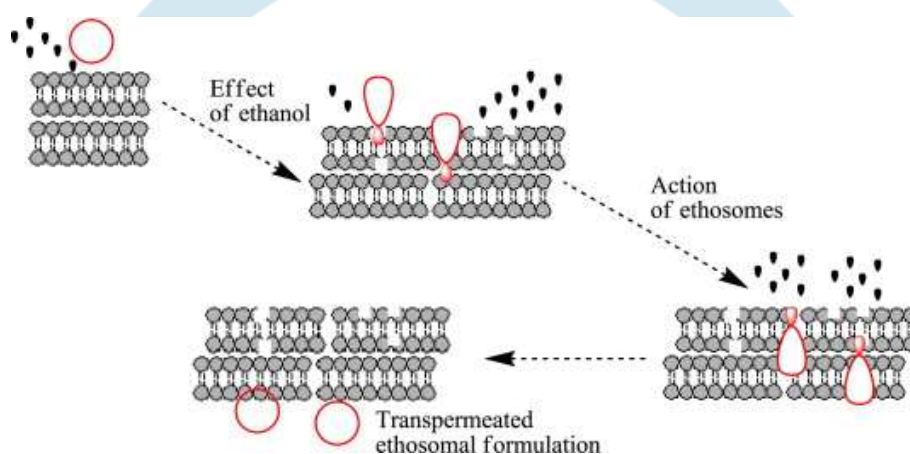


Figure 2: proposed mechanism for skin delivery of ethosomal system

Materials

Nabumetone was gift from Hetero Drugs(Hyderabad), Alcohol was purchased from Research lab fine chem industries (Mumbai), Carbopol 934 was purchased from Hetero Drugs(Hyderabad), Triethanolamine was purchased from SD fine chemicals(Mumbai), Propylene glycol was purchased from SD fine chemicals(Mumbai). The water used for all experiments was double distilled and filtered before use through nylon paper.

Preparation of sonicated nabumetone ethosomes(by cold method):

The Nabumetone ethosomal system comprised of 20-40% of ethanol, 10% of propylene glycol, 2-7% of phospholipids, 0.005 g of cholesterol and an aqueous part of 100 % w/v. At room temperature, 0.025g of Nabumetone was added to ethanol in a covered vessel along with propylene glycol, and dissolved by stirring vigorously. At 30°C mixture was heated using separate vessel and then water is added dropwise, the mixture in the center of the vessel by stirring it at 700 rpm for 5 min in a vessel. Then by using the extrusion method or sonication method the particle size of ethosomal formulation was reduced to desirable extent. At last ethosomal formulation is kept under refrigeration.^{5,6}

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Phospholipids + Drug
↓
Dissolve in ethanol
↓
Add propylene glycol
↓
Mixture is heated at 30±1°C
↓
Double distilled water is added with constant stirring for 5 min
↓
Vesicles size is controlled by using sonication or extrusion method
↓
Store under refrigerator

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Preparation of unsonicated nabumetone ethosomes:

The ethosomes consisted of 2-7% of phospholipids, 20-40% of ethanol, 0.005% of cholesterol, 0.025g of Nabumetone and distilled water quantity sufficient to 100 % w/v, the mixture was then heated at 30°C with continuous stirring at 700 rpm for 5 min and then transferred dropwise it another vessel kept closed.^{5,6}

Incorporation into gels

Carbopol 1% w/v was soaked in the mini amount of water for an hour, 20 ml of ethosomal suspension of nabumetone was added to the swollen polymer under continuous stirring at room temperature of 30°C until homogeneous gels were achieved. The pH was then adjusted to neutral using Triethanolamine and stirred slowly until a clear transparent gel was obtained.⁷

Characterization of preparing ethosomal formulation:

Vesicle size: The size of the vesicles can be characterized by optical microscopy with a calibrated stage micrometer.⁸

Zeta potential: particle size of vesicles can be determined by dynamic light scattering(DLS). The charge of the ethosomal vesicle is an important parameter that can influence both vesicular properties such as skin-vesicle interactions, stability, and zeta potential which can be determined using a computerized inspection system(Malvern Zetamaster ZEM 5002, Malvern UK).The Particle size of ethosomes was also determined by using a particular software “particle size analysis” which was developed by BIOVIS. This special software is working on micro-photographs images with standard dimensions.⁹

Entrapment efficiency: Separation of un-entrapped drug and evaluation of entrapment efficiency can be measured by ultracentrifugation. The Separation of supernatant layer was done and diluted with a suitable quantity of water and absorbencies for the respective concentration of drug were analyzed.The entrapment efficiency was calculated using formula.

$$\text{Entrapment efficiency} = \frac{(T-C)}{T} \times 100$$

Where, “T” is total amount of drug detected both in supernatant layer and residual layer and “C” is the amount of drug detected only in the supernatant.¹⁰

Scanning electron microscopic studies(SEM): The SEM studies was also conducted to characterize the surface morphology of the ethosomal vesicles for which a drop of ethosomal system was mounted on clear glass stub, air dried and coated with Polaron E 5100 Sputter coater (Polaron,UK) and visualized under scanning Electron Microscope (SEM Leo 430,England).¹¹

In-vitro diffusion study using cellophane membrane:

The experiments were conducted in Franz diffusion cells having donor compartment and a receiver compartment. A suitable size of pre-treated cellophane membrane was mounted between donor and receptor cells of Franz diffusion cells(locally fabricated). The receptor contained 15ml of phosphate buffer solution (PBS) at pH 7.4, was constantly stirred by magnetic stirrer at 100 rpm and was maintained at a temperature of 37 ± 0.5 °C throughout the experiments. Formulation equivalent to 1gm of nabumetone ethosomal gel was applied homogeneously in the donor compartment; 1ml of sample were withdrawn from receiver at predetermined time intervals over 24 hours and replaced with an equal volume of fresh PBS.samples were analyzed for the drug content using spectrophotometer at 249nm.¹²

Rate release Kinetics: The results of in-vitro release profile were designed in modes of data treatment as follows:-

- ✓ **Zero-order kinetic model-** cumulative percentage drug released versus time is plotted.
- ✓ **First-order kinetic model-** Log cumulative percentage drug remaining versus time is plotted.
- ✓ **Higuchi's model-** Cumulative percentage drug released versus square root of time.
- ✓ **Korsmeyer equation/Peppas's model-** Log cumulative percentage drug released versus log time is plotted.¹³

In-vitro skin permeation studies using rat skin by Franz diffusion cell:

In-vitro skin permeation studies of ethosomal gel formulation were carried out on Franz diffusion cell with an effective diffusion area of 2.4cm² and 20 ml of receptor compartment capacity, using rat skin. Skin was excised from the rat, entire hair were removed and the dermal side of skin was wiped with isopropyl alcohol to remove adhering fat. Then, the skin was washed properly with distilled water. The skin was brought to room temperature and treated with 0.5 M NaOH solution for 1-1.5 hours.

Pre-treated skin was cut into appropriate size and mounted between the two half of Franz diffusion cell where dermis faced towards donor compartment and the stratum corneum faced towards donor compartment. Initially, receptor compartment was filled with phosphate buffer (pH 7.4) while donor compartment was kept empty. The temperature of apparatus was maintained 37 ± 0.5 °C throughout the study period. The receiver fluid was stirred with magnetic rotor at a speed of 100 rpm. The whole receiver fluid showed a negligible UV absorption after 3 hours indicating complete stabilization of the skin membrane. The formulation equivalent to 1gm of Nabumetone was applied on the skin in donor compartment. Sample were withdrawn at predetermined time intervals and suitably diluted with phosphate buffer to analyze the drug content.⁷

Stability studies

Stability studies was carried out by storing the ethosomal formulation at the two different temperatures i.e is 4°C and 25 ± 2 °C. The drug content is then estimated for 3 months to identify any changes in the entrapment efficiency of ethosomal formulation.⁷

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