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FORMULATION DEVELOPMENT AND CHARACTERIZATION OF AZOLE DERIVATIVE LOADEDETHOSOME AND LIPOSOME: A COMPARATIVE ASSESSMENT REPORT

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ABSTRACT
Skin act as a major target as well as a principle barrier for transdermal drug delivery. Vesicular system is one of the most promising approaches for transdermal delivery of active substances. Liposomes are most commonly used vesicular delivery system. But it has certain limitations such as lesser stability, reduced encapsulation efficiency, etc led to formulation of ethosomes. In the present work we encapsulated various concentration of Miconazole Nitrate (an antifungal drug) within ethosome and liposome and made a comparative evaluation of their morphology, size, potential, stability and anti-fungal efficacy. Ethosomes showed better stability, encapsulation efficiency and anti-fungal activity as compared to liposome due to its ethanolic content. So ethosomal formulation may prove as a very promising option for transdermal delivery and has potential for new opportunities for topical application of Miconazole Nitrate in the fungal infections.
KEY WORDS: *drug delivery, transdermal, encapsulation efficiency, topical, Miconazole Nitrate liposome, ethosome.*

INTRODUCTION

Nanotechnology is a multidisciplinary field that covers a vast and diverse array of devices derived from engineering, physics, chemistry, and biology (Sahoo.S.K., et al, 2006). It is the technique of manipulating matter at the scale of atom and molecules (Drexler et al., 1986). In 1974 Norio Taniguchi used the phrase "nanotechnology" for the first time while describing an ion sputter machine. The term "nano" which means "dwarf" was originally derived from Greek word. Nanotechnology works with materials, devices, and other structures with at least one dimension sized from 1 to 100 nanometres. Nanometre is defined as one billionth of a metre that is equivalent to the length of ten hydrogen atoms. Now a day's nanotechnology hasa global interest. As mentioned in Environmemt and green nano the term nanotechnology embraces various fields and specialities including green nanotechnology, wet nanotechnology, nanoengineering, nanobiotechnology. Application of nanotechnology in commercial products like nanomedicine and green technology minimizes energy consumption and enhances the environmental sustainability of processes currently producing negative externalities, thus increasing the efficiency of energy production or quantum aged atoms (QCAs) (Robert A. Freitas Jr. 1999). Nanotechnology also has numerous potential application in the fields of consumer goods, providing with products with novel functions ranging from easy to clean to scratch-resistant (Neethirajan et.al., 2009) .

The structural and functional unit of nanotechnology is nanoparticle. It is defined as a small object that behaves as a whole unit with respect to its transport and properties. According to their diameter particles are further classified into three categories.(Grangvist et al., 1976).

- Coarse particles (10,000-2,500 nm)
- Fine particles (100-2500 nm)
- Ultrafine particles (1-100 nm)

Various types of nanoparticles are present which includes Nanosphere, Nanocapsule, Dendrimer, Polymeric micelles, Liposome and SLN (solid lipid nanoparticles). Nanosphere are considered as a matrix system in which matrix is uniformly dispersed. Beside of these spherical vesicular system is known as nanocapsules. In case of polymeric nanoparticles, the polymeric membrane surrounds the drug

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in a matrix core. Mostly biodegradable polymers are used in polymer nanoparticles likes polycyanoacrylate or poly (D, L-lactide) and related polymers like poly(lactic acid)PLA or poly(lactide-co-glycolide) etc. Dendrimers is a unique class of polymers which is highly branched macromolecules whose size and shape can be precisely controlled. Application of nanoparticles target drug delivery, drug bio-availability, detection of pathogen etc (Abhilash M,. 2010).

Phospholipids are the major components of all cell membranes as they form lipid layers. Phospholipid is a class of lipid mainly consisting of diglyceride and phosphate group. The structure of the phospholipid molecule consists of hydrophobic tails and a hydrophilic head. Lecithin or phosphatidylcholine from egg yolk was the first identified phospholipid. Other common phospholipids are phosphatidic acid (phosphatidate), phosphatidyethanolamine (cephalin), phosphatidylcholine (lecithin), phosphatidylserine, phosphoinositides, etc. Phospholipid synthesis occurs in the cytosol adjacent to endoplasmic reticulum (ER) (E Fahy et al., 2009).

Lecithin (phosphatidylcholine) is generic term which is used to designate yellow brownish fatty substance present in animal or plant tissue composed of phospholipids, phosphoric acid, triglyceride, glycolipids, etc. Lecithin is easily extracted from sources such as soybeans, eggs,milk, marine sources, rapeseed, cottonseed and sunflower chemically by using hexane, ethanol, etc. Lecithin has emulsification and lubricant properties. Lecithin from soybean and egg play an important role in drug delivery.

Liposome is an artificial microscopic single vesicle consisting of an aqueous core enclosed in one or more phospholipid layers used to convey vaccines, drugs, enzymes, or other substances to target cells or organs (Lawerence D, 1986). Liposomes are a form of nanoparticle prepared from lecithin. They are microscopic, concentric bilayered vesicles. Here the aqueous volume is entirely enclosed by a membraneous lipid bi-layer composed of natural or synthetic phospholipids. The major types of liposomes are multilamellar vesicles, small unilamellar vesicles, large unilamellar and cochleate vesicles (Sharma.A and Sharma U.S, 1997). Liposomes increase the efficiency, bioavailability, absorption of certain entrapped dietary and nutritional supplements and are used as topical drug delivery system. (C.George et al., 1975). But liposomal drug delivery system has certain shortcomings like the need for modification for site specific or organ specific drug delivery, high production cost and leakage and fusion of encapsulated drug/molecule.

ETHOSOME

Ethosomes were specially tailored novel vesicular carriers introduced by Touitou in the year 2000 (25). These are soft, flexible vesicle mainly composed of phospholipids, relatively high amounts of ethanol (20-45%) and water (Figure 6). The ethanolic vesicles differ from theconventional liposomes by a number of essential characteristics such as vesicle bilayer flexibility, its mechanism of enhanced skin permeation, easy method of preparation; stability andlack of toxicity. These systems have the capability to convey the drugs into the deeper skin layers to the extent of the systemic circulation. The high ethanol concentration (20-45%) imparts a negative surface charge to the vesicle which makes the size of the vesicles to decrease (26).

Advantages of Ethosomes

Ethosomes as novel lipid carriers offer numerous advantages over the other vesicularsystems (26, 27). These include

- Prepared with biodegradable and biocompatible components
- Contains non-toxic raw material in a formulation.
- Enhanced permeation of drug through the skin for transdermal drug delivery.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary,Cosmetic fields.
- High patient compliance: The ethosomal drug is administrated in semisolid form (gel or cream)hence producing high patient compliance.
- The simple method for drug delivery in comparison toIontophoresis and Phonophoresis andother complicated methods.
- The Ethosomal technology is available for immediate commercialization.
- Capability to efficiently encapsulate a wide variety of drug molecules including water-soluble,lipophilic, amphiphilic and high molecular-weight compounds.
- Provides sustained and controlled delivery of drugs.
- Provide better accumulation of drug molecule in the deeper layers of the skin and acts as depotsystems.
- Provides passive and non-invasive means of application.
- Improve the delivery of drug through skin under occlusive and nonocclusive conditions.
- Easy to scale up and vast market potential.

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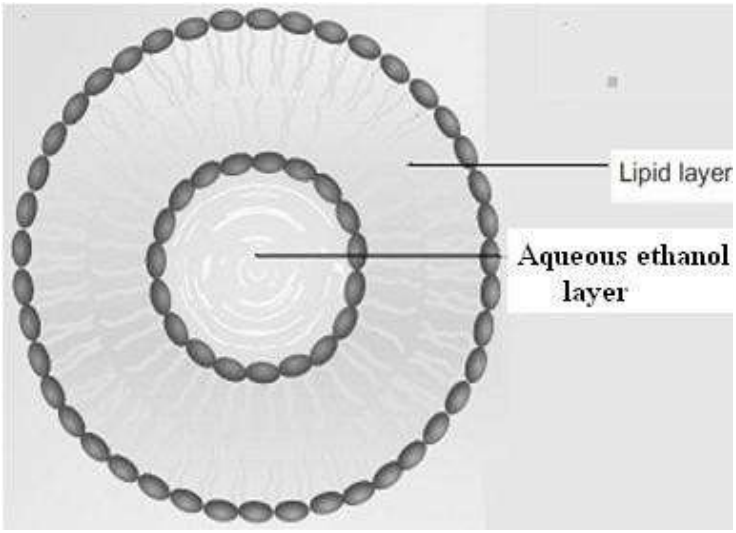

Figure . Schematic presentation of ethosomes

PREPARATION AND CHARACTERIZATION

❖Preparation of Ethasomes:

Selection of Phospholipids: Choose a suitable phospholipid(s) based on the desired characteristics and compatibility with the drug or active ingredient. Commonly used phospholipids include phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine.

Solvent Selection: Ethanol is the primary solvent used for ethosome preparation, as it enhances the flexibility and fluidity of the phospholipid bilayers. It also helps in solubilizing both hydrophilic and lipophilic drugs. Other solvents like propylene glycol or isopropyl alcohol can also be used in combination with ethanol to achieve the desired properties.

Drug Loading: Dissolve the drug or active ingredient in the appropriate solvent mixture containing ethanol. Ensure that the drug is completely solubilized or dispersed within the solvent.

Preparation Method: Several methods can be used to prepare ethasomes, including:

a. Thin Film Hydration Method: In this method, dissolve the appropriate amount of phospholipids in a suitable organic solvent mixture containing ethanol. Evaporate the solvent under reduced pressure to form a thin film of the lipid mixture on the walls of a round-bottom flask. Hydrate the lipid film with an aqueous solution, followed by sonication or vortexing to obtain the ethasomes.

b. Hot Method: Dissolve the phospholipids and the drug in a suitable organic solvent mixture containing ethanol. Heat the mixture to a temperature above the phase transition temperature of the phospholipids, followed by sonication or vortexing. Allow the mixture to cool to room temperature to obtain the ethasomes.

c. Cold Method: Dissolve the phospholipids and the drug in a suitable organic solvent mixture containing ethanol at room temperature. Place the mixture in an ice bath and subject it to sonication or vortexing until ethasomes are formed.

- **Characterization of Ethasomes:**Particle Size Analysis: Determine the size distribution of ethasomes using techniques such as dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), or laser diffraction. These techniques can provide information about the mean particle size, polydispersity index, and zeta potential of the ethasomes.

Morphology Analysis: Use transmission electron microscopy (TEM) or scanning electron microscopy (SEM) to visualize the morphology of ethasomes. These techniques can reveal the shape, structure, and surface characteristics of the vesicles

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Encapsulation Efficiency: Determine the amount of drug or active ingredient encapsulated within the ethasomes. This can be done by separating the unencapsulated drug from the ethasomes using techniques such as centrifugation or dialysis, followed by quantification of the drug concentration using suitable analytical methods.

Stability Studies: Assess the physical and chemical stability of ethasomes over time. Study factors such as particle size changes, drug leakage, and vesicle aggregation under different storage conditions (temperature, light, etc.) over a specified period.

In vitro Drug Release Studies: Evaluate the release profile of the drug from ethasomes using suitable dissolution methods. Measure the amount of drug released at different time intervals to understand the release kinetics and release mechanism.

AIMS & OBJECTIVES

1. To prepare ethosomes and liposomes encapsulating Miconazole Nitrate in variousconcentrations.
2. To characterize the prepared ethosome and liposome.
3. To determine the efficacy of prepared ethosome and liposome against fungalpathogens.

MATERIALS & METHODS

I. MATERIALS

Chemicals Required

The drug Miconazole Nitrate was obtained from local pharmaceutical store under the brand name Monistat. Lecithin (Trade name-Leciva- S70) containing not less than 98% phosphatidyl- choline was received as a kind gift from VAV LIFE SCIENCES Pvt. Ltd, Mumbai, India. Ethanol and methanol was purchased from Hi-media Pvt. Ltd Mumbai, India.Distilled water and all other chemicals and solvents used in our work were of analytical gradeand available in Department of Pharmaceutics, GRKIST PY, Jabalpur, M.P.

Instruments Used

The basic instruments used for the preparation and characterization of the samples likeWeighing balance (Contech-CA223, 2008), Magnetic stirrer (Remi), Probe Sonicator (Plexiglas), Refrigerated centrifuge (Thermofisher), UV Spectrophotometer (Agilent technologies cary 60, 2012), FT-IR (Agilent technologies cary 630, 2012) was available in the Department of Pharmaceutics, GRKIST PY, Jabalpur, M.P.

For Specialised assessment like Scanning Electron Microscopy, Atomic Force Microscopy and Particle Size Analyzer samples were sent to Diya labs Mumbai and STIC cochin.

II. PREPARATION OF STOCK SOLUTION OF MICONAZOLE NITRATE

Miconazole Nitrate stock solution was prepared by dissolving 1mg drug in 10 ml ethanol according to the manufacturer's instruction. Working solutions of different concentrations ranging from 0.3125µg/ml to 30 µg/ml were prepared from the stock solution.
When water was used to dissolve the drug, it resulted in immediate precipitation of the drug, so the ethanol was chosen as a solvent

PREPARATION OF ETHOSOME AND LIPOSOME

Preparation of blank ethosomal particle

Ethosome was prepared by solvent dispersion method as described by Touitou et.al. 2000. Briefly Lecithin (up to 2-3%), was dissolved in (30-40%) of 90% ethanol by use of magnetic stirrer (Remi Motors Mumbai) for 20 minutes at 100 rpm. To this mixture warm distilled water was slowly added in a fine stream by syringe and the whole system was stirred for 30 minutes at 700 rpm. The resulting preparation was sonicated for 3 cycles of 5 minute each with 5 minute rest between the cycles.

Preparation of drug (Miconazole Nitrate) encapsulated ethosomes

Miconazole Nitrate (drug) encapsulated ethosomes were also prepared by solvent dispersion method following the protocol by Touitou et.al, 2000. Lecithin (2-3%) and Miconazole Nitrate was dissolved in (30-40%) of 90% ethanol by use of magnetic stirrer (Remi Motors Mumbai) for 20 minutes at 100 rpm. Then warm distilled water was added slowly in a fine stream to the ethanolic drug mixture solution and the mixture was stirred for 30 minutes at 700 rpm in a closed vessel. The resulting preparation was sonicated for 3 cycles of 5 minute each with 5 minute rest between the cycles. This method was repeated several times but by varying the drug concentration each time.

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Preparation of blank liposomal particle

Liposomes were prepared by classic dispersion method as described by Touitou et.al. 2000 with certain modifications. Lecithin (2-3%) was dissolved in 6 ml distilled water. This mix was heated to 30◦C in a water bath. To this mixture warm distilled water was added slowly in a fine stream with continuous stirring at 700 rpm in close vessel. The resulting vesicles were sonicated for 3 cycles of 5 minute each with 5 minute rest between the cycles.

Preparation of drug (Miconazole Nitrate) encapsulated liposomes:-

Miconazole Nitrate (drug) encapsulated liposomes were prepared as described by Touitou et.al, 2000 with little modification of the classic dispersion method. Briefly lecithin (2-3%) and drug solution was taken and dissolved in 6 ml distilled water and this mix was heated to 30◦C in a water bath. Warm distilled water was added slowly in a fine stream to the drug-lipid suspension with continuous stirring at 700 rpm in a closed vessel. The resulting vesicles weresonicated for 3 cycles of 5 minute each with 5 minute rest between the cycles.

RESULTS

The various formulations of liposomes and ethosomes were characterized and their results shown below.

Vesicle Morphology

The various drug loaded formulations of ethosomes and liposomes (including the blank particles) appeared more or less spherical when observed by SEM (Fig. 1).

Particle size and particle size distribution

The particle size, zeta potential and size distribution of the various suspensions are shown in Table 4.1. The zeta potential of all the ethosomal vesicles was of higher magnitude than liposomal vesicles (measured in milivolts). The poly index measured in PDI and particle size measured in nanometre.

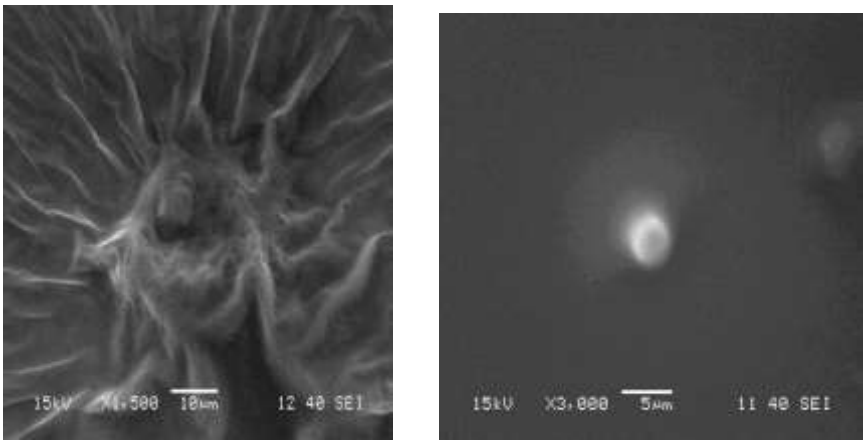
Drug Entrapment Efficiency

The entrapment efficiency was calculated as mentioned in Chapter 3. The quantity of drug entrapped is more in case of ethosome as compared to liposome. Among the various formulations when drug concentration is around 20-30 µg/ml, the quantity of drug entrapped is more. The high ethanol concentration favoured better encapsulation. The values of encapsulation efficiency is shown in Table 4.4.

Antifungal Studies

The in-vitro antifungal studies qualitatively showed the better efficacy of drug loaded ethosomes against the drug loaded liposomes. The blank formulations did not show any activity against the fungal pathogens. The results are shown in Fig.4.13.

Fig 4.1.Concentration of drug 5µg/ml



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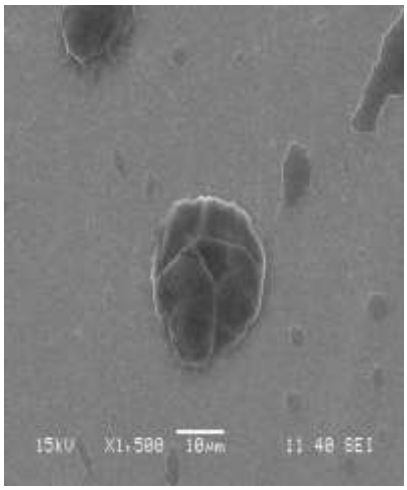
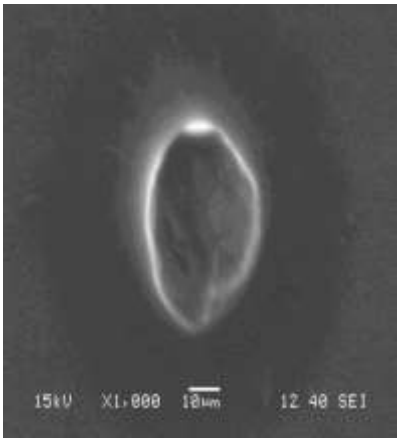
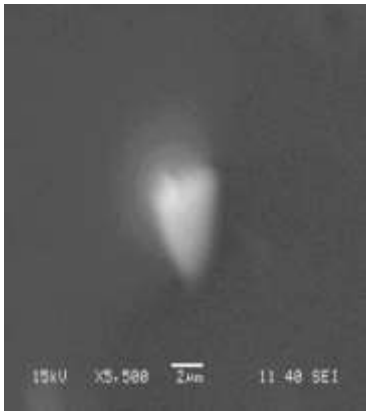
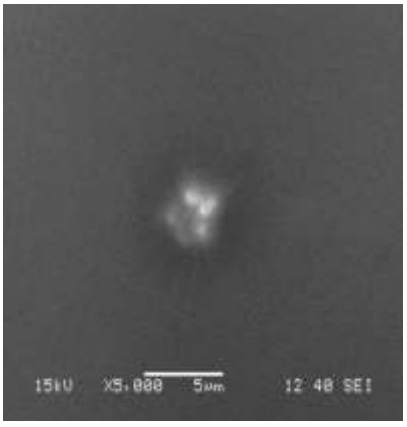


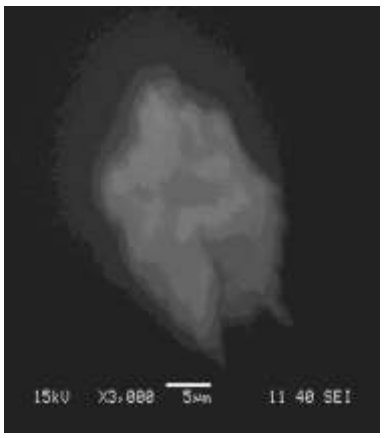
Liposome



Ethosome







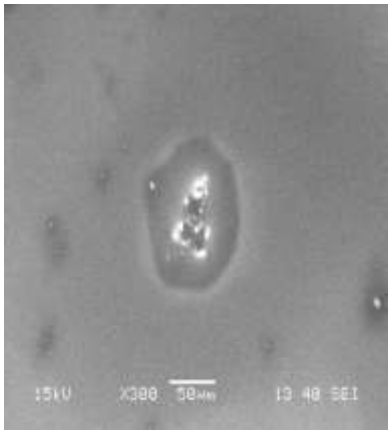


Fig 4.2. Concentration of drug 10µg/ml
Fig 4.4. Concentration of drug 30µg/ml
Fig 4.3.Concentration of drug 20µg/ml

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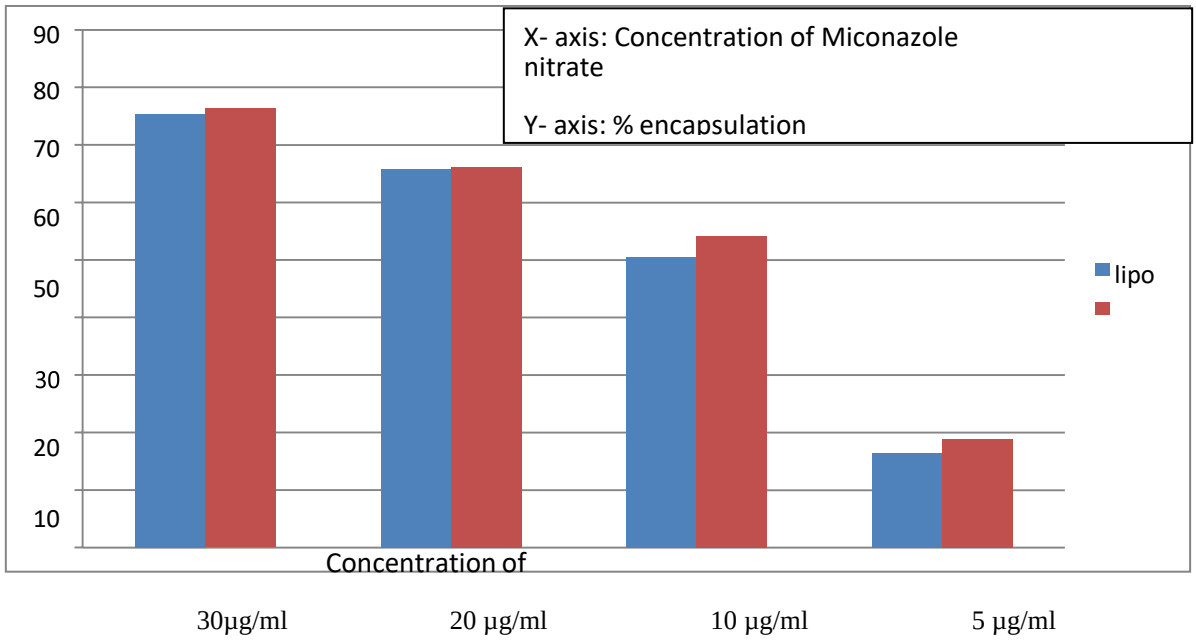
Fig 4.15. Graph showing comparative encapsulation efficiency of ethosome and liposomeat various drug concentrations



DISCUSSION & SUMMARY

Development of novel drug delivery carriers are a necessity to deliver the drugs to target site at a faster rate to overcome the drawbacks of multi-dose therapy and increase patient compliance and improve their safety. For drug delivery via dermal and transdermal routes ethosomes have emerged as a non-invasive mean.

CONCLUSION

The ethosomal formulations were more spherical with stable zeta potential and mono- disperse with no clumping. Although the liposomes showed spherical morphology but were less stable and usually poly-dispersed in nature. The antifungal activity of liposome was less as compared to ethosomes.

So from the study it was confirmed that ethosomal formulation of Miconazole Nitrate showeda good entrapment efficiency and better stability profile as compared to liposomes. Thus it is concluded that ethosomal formulation is a very promising option for transdermal delivery andhas potential for new opportunities for topical application of Miconazole Nitrate in the fungal infections.