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DEVELOPMENT AND CHARACTERIZATION OF OXICONAZOLE NITRATE LOADED ETHOSOMAL GEL FOR TREATING FUNGAL INFECTIONS

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ABSTRACT

The present investigation includes development and characterization of oxiconazole nitrate loaded ethosomal gel for treating contagious diseases. Topical therapy permits administration of small amount of oxiconazole nitrate, which is directly applied on to the skin such that it penetrates through the skin and reaches the specific site to show its pharmacological action efficiently as in this route of drug delivery first pass metabolism is bypassed and by this route of drug delivery and more than 70% bioavailability was achieved. Effect of different formulations on characterization and *in-vitro* drug release was conducted and ethosomes containing ethanol showed better entrapment efficiency, small vesicle size, high transdermal flux and maximum skin penetration. One basic and advantageous methodology is use of medications in plan with versatile vesicles or skin enhancers. Significant enhanced delivery of oxiconazole nitrate through transdermal route could be obtained by using vesicular drug carrier systems - Ethosomes. The present examination was meant to explore the capability of ethosomes in upgrade of oxiconazole nitrate transport over the skin, qualities of ethosomes and their in-vitro skin pervasion conduct. The present work also focuses on making the formulation more pharmaceutically acceptable.

KEYWORD: Ethosomes, Transdermal Drug Delivery, Oxiconazole Nitrate, Fungal Infections.

INTRODUCTION

India is a country with vast diversity in the climatic and weather conditions. The climate is generally hot, humid and tropical type. This type of climate tends to favour them growth of variety of microorganisms and disease causing pathogens. Many studies have reported the occurrence of various diseases and infections caused by such organisms. About 10% of annual funding for health research is spent on health problems that account for 90% of global disease burden.[1] Fungal infection is one such disease common among the Indians. It has been observed that people tend to neglect the fungal infections greatly and majority doesn't consider it as a disease and prefers to go for unconventional treatments. Around the world fungal infections have recently emerged as a growing threat to human health.[2]

Fungi generally shows growth in humid and moist parts of the body where skin surfaces meet such as in the genital area, under the breasts and between the toes. A lot of fungi that infect the skin (dermatophytes) live only in the uppermost layer of the epidermis (Stratum corneum) and do not penetrate deeper. The penetration of drugs through the target tissue tells about the efficacy of the topical antifungal drugs. Therefore, it is required to achieve an efficient drug concentration levels in the skin. The antifungal drugs should be capable to pass the stratum corneum (outermost layer of the skin) to reach the lower layers of the skin, chiefly into the viable epidermis.

Ethosomes

Ethosomes are slight modification of well established drug carrier liposome. Ethosomes are lipid vesicles containing phospholipids, alcohol in relatively high concentration and water. The size range of ethosomes may vary from tens of nanometers to few microns. Ethosomes permeate through the skin layers more rapidly and possess significantly higher transdermal flux.

Vesicles would also allow to control the release rate of drug over an extended time, keeping the drug shielded from immune response or other removal systems and would be able to release just the right amount of drug and keep that concentration constant for longer periods of time.[3]

Ethosomal carriers are systems containing soft vesicles and are composed mainly of phospholipid (Phosphotidyl choline; PC), ethanol at relatively high concentration and water.

It was found that ethosomes penetrate the skin and allow enhanced delivery of various compound to the deep strata of the skin or to the systemic circulation Fig. 1.

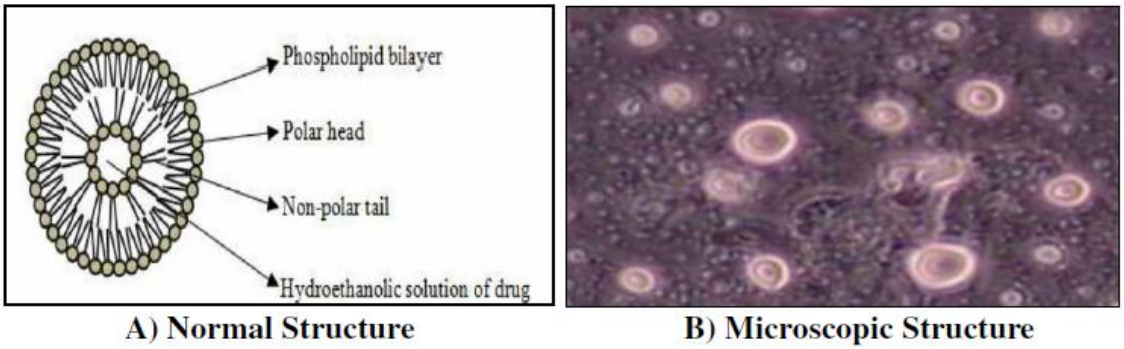

Fig. 1: Structure of Ethosomes.

Mechanism of Penetration

Although the exact process of drug delivery by ethosomes remains a matter of speculation, high concentration of ethanol makes the ethosomes unique, as ethanol is known for it's disturbance of skin lipid bilayer organization; therefore, when integrated into a vesicle membrane, it gives that vesicles have the ability to penetrate the stratum corneum. Ethanol interacts with lipid molecules in the polar hard group region, resulting in a reducing the rigidity of the stratum corneum lipids, increasing their fluidity. The intercalation of ethanol into the polar head group environment can result in an increase in the membrane permeability. In addition to the effect of ethanol on stratum corneum structure, the ethosome itself may interact with the stratum corneum barrier.[4]

MATERIALS AND METHODS

Oxiconazole nitrate was procured from Yarrow Chem Products, Mumbai, India. Carbopol 934, HPMCK 4M, Chloroform and Methyl Paraben was procured from S.D. Fine Chem. Ltd, Mumbai, India. Ethanol was obtained from Qualigens Fine Chemicals Mumbai, India All other reagents used were analytical grade.

Formulation Of Ethosomes And Drug Loaded Ethosomal Gel

Preparation of ethosomes

Ethosomes were prepared by hot method using lipid soya lecithin. Lipid and cholesterol in different ratios were accurately weighed and dissolved in water and kept for stirring using magnetic stirrer for 30 min with heating at 40 ºC. Organic phase containing specified amount of Oxiconazole nitrate was added to ethanol and to this propylene glycol was added and kept

for stirring separately. Then, lipid solution was added drop by drop to the organic phase and kept for stirring on a magnetic stirrer for 1 hr. The solution was subjected to sonication using probe sonicator for 15 min to reduce the particle size.[5,6]

Preparation of Drug Loaded Ethosomal Gels

The gels were prepared by dispersion method using HPMCK4M and Carbopol 934 in different ratios. Gels were prepared by dispersing gelling agent to the distilled water. Then the mixture was allowed to swell overnight. The mixture was neutralized by drop wise addition of triethanolamine. Then, glycerol was added to gel to balance its viscosity. To this gel solution optimized ethosomal dispersion was added and mixed properly. Mixing was continued until a transparent gel appears. Methyl Paraben was added as a preservative. The prepared gels were filled in glass vials and stored at 4-8^{0}C.[7]

Characterization of Ethosome Vesicles[8]

Visual Examination (Optical Microscopic Observation): For visual examination ethosomal dispersion was first spread on the glass slide using a glass rod. Formation of multi lamellar vesicles was confirmed by examining the ethosomal suspension under an optical microscope (Olympus) with a magnification of 40x and 10x.

Particle Size and Size Distribution

1 ml of Ethosome was diluted to 10 ml with distilled water and average particle size and polydispersity index were measured by using a Zetasizer (Malvern, UK). The particle size and polydispersity index was found to be 99.5nm and .405 respectively.

Drug Entrapment Efficiency

Entrapment efficiency of Oxiconazole nitrate ethosomal vesicles was determined by centrifugation. The vesicles were separated in a high speed cooling centrifuge at 20,000 rpm for 90 minutes. The sediment and supernatant liquids were separated, amount of drug in the sediment was determined by lysing the vesicles using methanol. It was then diluted appropriately and estimated using UV visible spectrophotometer at 427 nm. From this, the entrapment efficiency was determined by the following equation.

$$EE\% = \frac{\text{(Total drug) - (free drug) X 100}}{\text{Total drug}}$$

In-Vitro Drug Release

The *in-vitro* drug release studies of Oxiconazole nitrate from ethosomal formulation were studied using locally modified diffusion cell. The in-vitro diffusion of the drug was performed through one end of the hollow glass tube of 17 mm (area 2.011cm^2). This acted as donor compartment. 50 ml of Phosphate buffer saline 7.4 was taken in a beaker which was used as a receptor compartment. A known quantity was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.5ºC. The solutions of the receptor side were stirred by small magnetic bead and were rotated at a constant speed.

At predetermined time intervals, samples were withdrawn and replaced by 5ml of PBS. The drug concentrations in the aliquot were analysed for drug content using UV spectrophotometer (Lab India UV 3000+) at 427 nm against appropriate blank. The optimized ethosomal formulation was taken and incorporated in to gel.

Characterization of Oxiconazole nitrate Loaded Ethosomal Gel[9-12]

Appearance

The appearance was checked visually. After gelling, the clarity and colour of the formulations was determined by visual examination of the formulations under light, alternatively against white and black background.

pH

pH was checked using pH meter (Hicon, India). The electrode was submersed into the formulation at room temperature and the readings were noted.

Drug content

Drug content was estimated spectrophotometrically. 50 mg equivalent of gel was taken and dissolved in methanol and filtered. The volume was made up to 10 ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 427 nm.

Viscosity

Viscosity has significant role in the performance of topical products. Viscosity of formulation is closely linked to the product characteristics, such as spreadability, ease of application, drug release and stability. Viscosity was determined by Brookfield viscometer (Ametek

Brookfield) and the angular velocity was found to be increased from 5, 10, 25, 50, 100 rpm and the values were noted.

Extrudability

In conducting the test, a closed collapsible tube containing above 20 grams of the gel was pressed firmly at the crimped end and a clamp was applied to prevent any rollback. The cap was removed and the microcapsule-containing gel was extrudes until the pressure was dissipated.

Spreadability

Spreadability of gel was determined by modified wooden block and glass slide apparatus. A measured amount of gel was placed on fixed glass slide; a movable pan with a glass slide attached to it and was placed over the fixed glass slide, such that the gel was sandwiched between two glass slides for 5 minutes. The weight was continuously removed. Spreadability was determined using the formula:

S= M/T

Where S, is the spreadability in g/s, M is the mass in grams & T is the time in seconds.

In-Vitro Drug Release

The in-vitro drug release studies of oxiconazole nitrate loaded ethosomal gels were studied using locally modified diffusion cell. The in-vitro diffusion of the drug from ethosomal gel was performed through one end of the hollow glass tube of 17 mm (area 2.011cm^2). This acted as donor compartment. 50 ml of Phosphate buffer saline 7.4 was taken in a beaker which was used as a receptor compartment. A known quantity was spread uniformly on the membrane. The donor compartment was kept in contact with the receptor compartment and the temperature was maintained at 37±0.5ºC. The solutions of the receptor side were stirred by small magnetic bead and were rotated at a constant speed. At predetermined time intervals, samples were withdrawn and replaced by 5ml of PBS. The drug concentrations in the aliquot were analysed for drug content using UV spectrophotometer (Lab India UV-3000+) at 427 nm against appropriate blank.

Anti Fungal Studies

After preparation and sterilization of sabouraud dextrose agar medium at room temperature was inoculated with candida albicans (fungal strain) and then the medium was poured into the

three Petri dishes and allowed to cool it for some time at room temperature until it solidifies and then three cups were bored in each Petri dish with the help of sterile bore of 6mm diameter and calculated concentration of the commercial Oxiconazole nitrate cream (Oxistat), gel formulations EG-1, and free Oxiconazole nitrate gel (prepared without ethosomes) were placed in the bores and the Petri-plates were incubated at 37^0C for 72 hrs in incubator. The zone of inhibition was observed and the radius of the zone of inhibition was calculated.

RESULTS AND DISCUSSION

Formulation of Ethosomes

The formula for formulation of ethosome by hot method with sonication and their results are shown in **table 1**. In this technique the particles were found to be smaller than micron range, the shape was spherical, and no aggregation took place.

Table 1: Formulations of Oxiconazole nitrate loaded Ethosomes.

Formula Code	Drug concentration (mg)	Lecithin (mg)	Cholesterol (mg)	Ethanol (ml)	Propylene glycol (ml)
F1	100	100	25	10	3
F2	100	200	25	10	3
F3	100	300	25	10	3
F4	100	400	25	10	3
F5	100	500	25	10	3
F6	100	300	25	10	3
F7	100	300	35	10	3
F8	100	300	45	10	3
F9	100	300	55	10	3
F10	100	300	65	10	3
F11	100	300	75	10	3
F12	100	300	85	10	3
F13	100	300	45	15	3
F14	**100**	**300**	**45**	**20**	**3**
F15	100	300	45	25	3
F16	100	300	45	30	3
F17	100	300	45	35	3

Characterization of Ethosomes

Visual examination

The visual examination of ethosomes was confirmed by using optical microscope and the results are given below in figure 2.

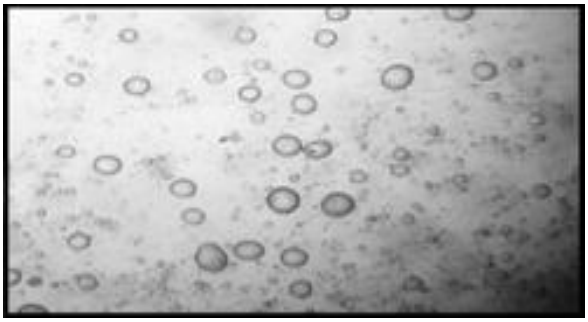

Fig. 2: Microscopic image of Ethosomes.

Particle Size and Size Distribution

The particle size and polydispersity index was found to be 99.5nm and -405 respectively. The related report is given in figure 3.

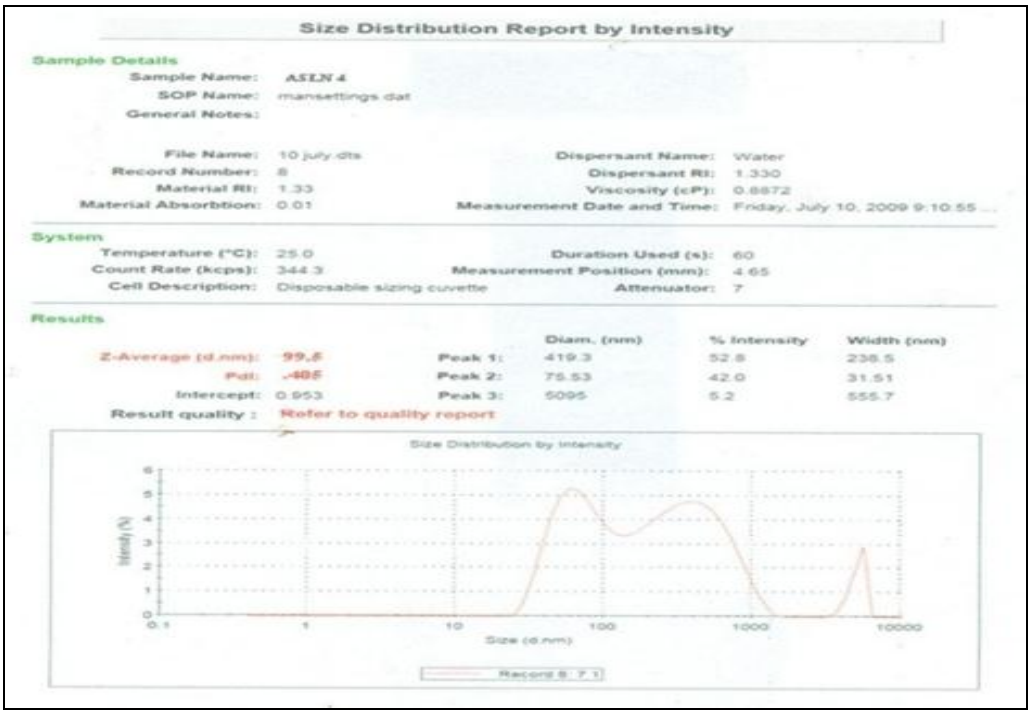

Fig. 3: Particle Size and Size Distribution Report of Ethosome.

Drug Entrapment Efficiency

The Drug entrapment efficiency of all formed formulation of Oxiconazole nitrate entrapped ethosomal vesicles was given in Figure 4. The drug entrapment efficiency of all ethosome formulations are found in range of 66% to 88.3% in which the Drug entrapment efficiency of F14 formulation was founded maximum among all i.e., 88.3%.

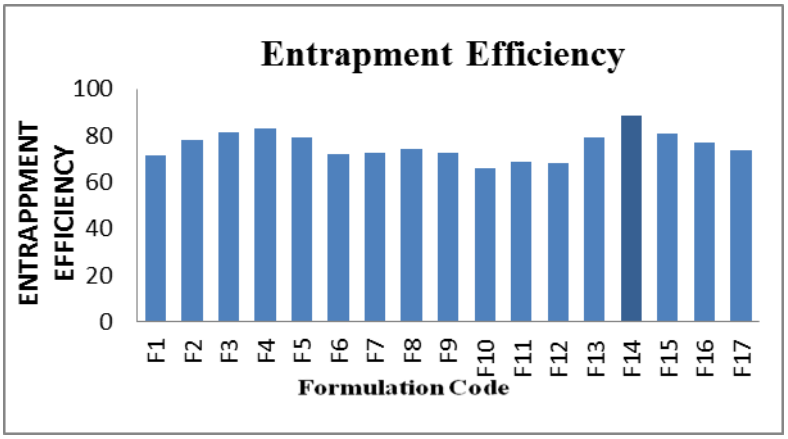

Fig. 4: Entrapment efficiency of ethosomes.

***In-vitro* drug release**

The cumulative percentage drug release from various ethosomal formulations is shown in fig 5, 6, and 7. Formulation F14 showed higher cumulative drug release of 85.3% in 8 hrs. It showed higher entrapment efficiency and drug release than other formulations. Therefore F14 has been selected for formulating the ethosomal gel.

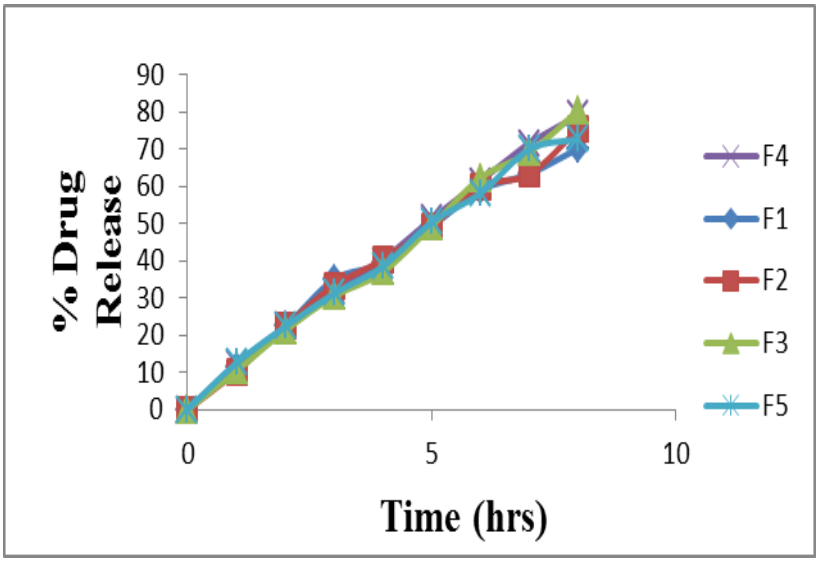

Fig. 5: *In-vitro* percentage drug release of ethosomes prepared by different concentrations of lecithin.

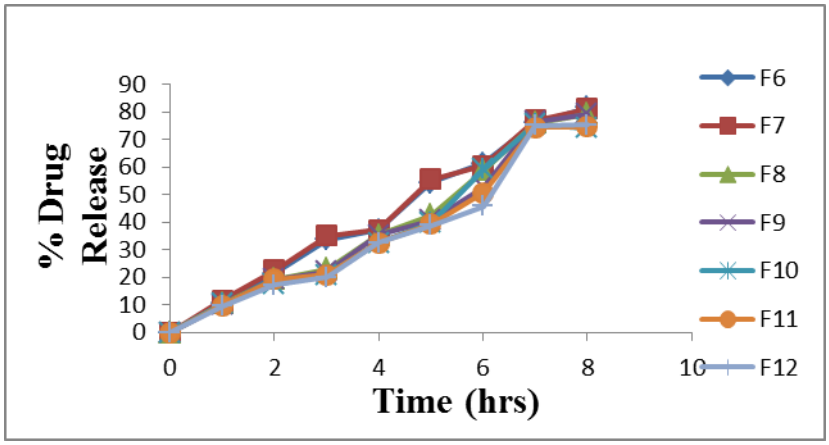

Fig. 6: In-vitro percentage drug release of ethosomes prepared by different concentrations of cholesterol.

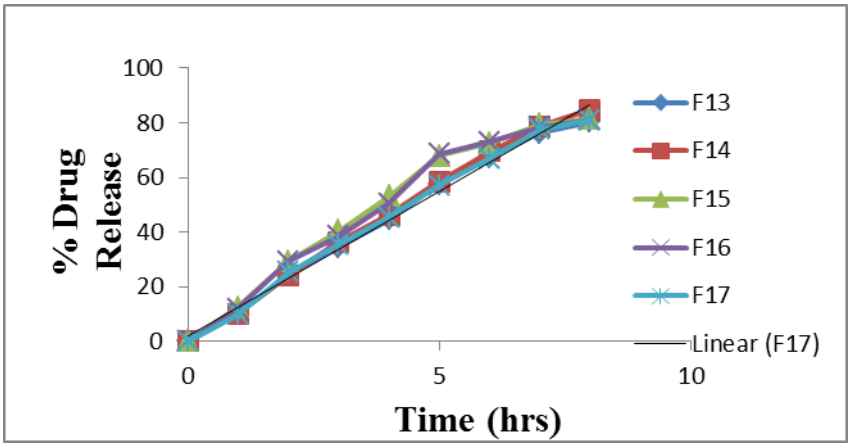

Fig. 7: In-vitro percentage drug release of ethosomes prepared by different concentrations of ethanol.

Formulation and Characterization of Oxiconazole Nitrate Loaded Ethosomal Gel

Formulation of Oxiconazole Nitrate Loaded Ethosomal Gel

The gels were prepared by dispersion method using HPMCK4M and CARBOPOL 934 in different ratios as shown in table 2.

Table 2: Formulation of ethosomal gels using Carbopol 934, HPMCK4M.

Formula code	Ethosomal suspension (ml)	Carbopol 934 (%)	HPMCK4M (%)	Propylene glycol (ml)	Triethanolamine (%v/v)	Methyl paraben (%)	Water (up to 30 gm)
EG1	10	0.5	-	5	0.5	0.01	q.s
EG2	10	1	-	5	0.5	0.01	q.s
EG3	10	1.5	-	5	0.5	0.01	q.s
EG4	10	-	0.5	5	0.5	0.01	q.s
EG5	10	-	1	5	0.5	0.01	q.s
EG6	10	-	1.5	5	0.5	0.01	q.s

Characterization of Oxiconazole Loaded Ethosomal Gel

The appearance was checked visually. After gelling the clarity of the formulations colour was determined by visual examination under light, alternatively against white and black background. The colour of ethosome is observed to be about pale yellow to colourless with translucent appearance.

The pH of formulations was in the range of 6.23 to 6.93 which considered acceptable to avoid the risk of skin irritation upon application to skin. The Result are shown in Figure 8. The optimized formulation (EG1) pH was found to be 6.85. There was no significant change in pH values as a function of time for all formulation. The drug content was estimated spectrophotometrically. 50mg equivalent of gel was taken and dissolved in methanol and filtered. The volume was made up to 10ml with methanol. The resultant solution was suitably diluted with methanol and absorbance was measured at 427nm.

The EG1 formulation shows maximum % Drug content i.e., 95.56% and thus selected as a final formulation. The results are shown in Figure 9. The average viscosity of formulations lies in the range from 937.459 to 5650.623 cps. The Viscosities of all gel formulations are shown in Fig 10 and 11 and was found to be decreased on increasing the shear rate i.e., pseudo plastic behaviour was noted.

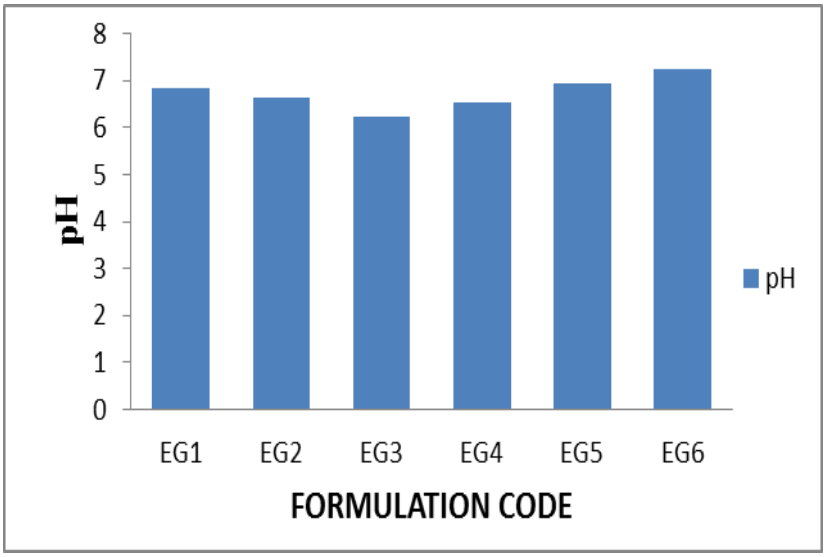

Fig. 8: pH of ethosomal gel formulation (EG1-EG6).

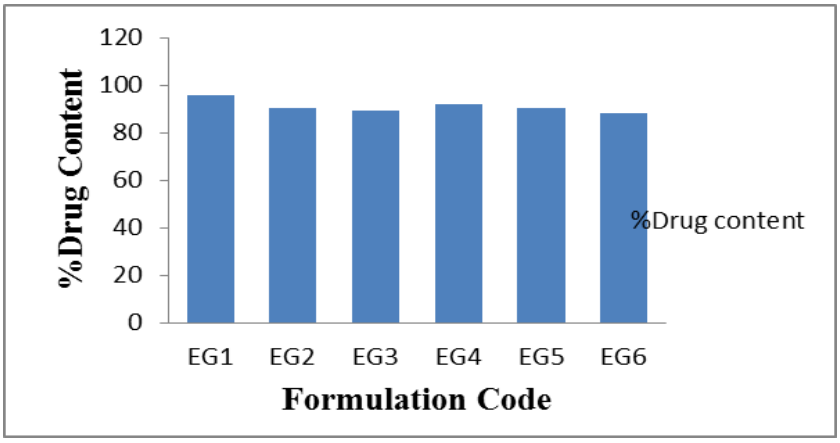

Fig. 9: % Drug content of ethosomal gel.

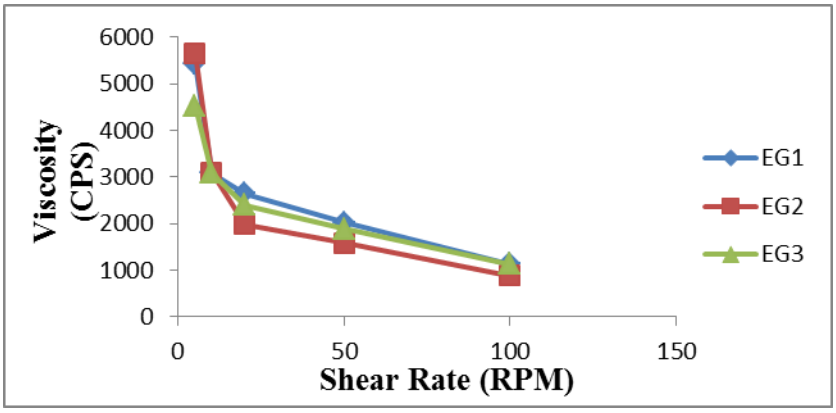

Fig. 10: Viscosity of Ethosomal gel Formulations EG-1 to EG-3.

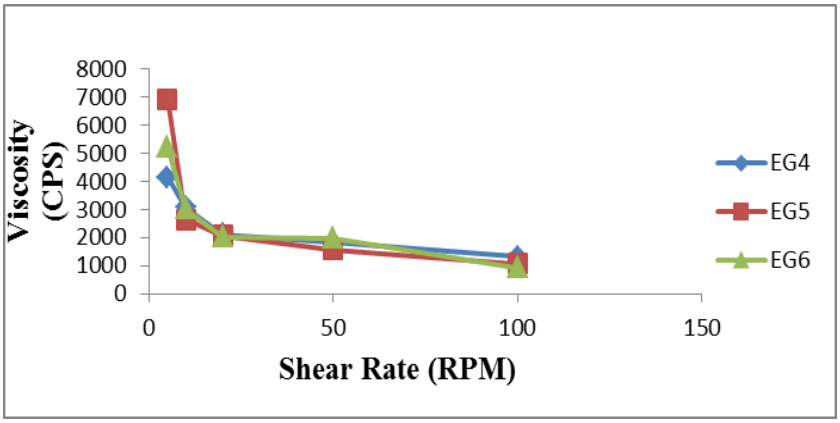

Fig. 11: Viscosity of Ethosomal gel Formulations EG-4 to EG-6.

The extrudability of formulations were determined as per the method mentioned using a weight of 20 gms. Most of the gels exhibited excellent extrudability characteristics. (+ fair, ++ Average, +++ Excellent). The observations of extrudability studies are given in Table 3. Ethosomal gels agent exhibited spreadability values ranging from 10.60-13.75 g.cm/s. The spreading coefficient of various ethosomal gel formulations are given below in table 3.

Table 3: Extrudability and Spreadability study of formulations.

Formulation	Extrudability	Spreadability
EG1	+++	13.75
EG2	++	13.54
EG3	+++	12.88
EG4	+++	11.48
EG5	++	10.60
EG6	++	11.10

Excellent: +++, Good: ++, Average: +, Poor: -

***In-vitro* drug release study**

The in vitro drug release studies were carried out across cellophane membrane. The results of in-vitro release after incorporation of ethosomes in gels are shown in fig 12 and 13. The cumulative percentage drug release for 8 hrs was highest for formulation EG1 using carbopol 934.

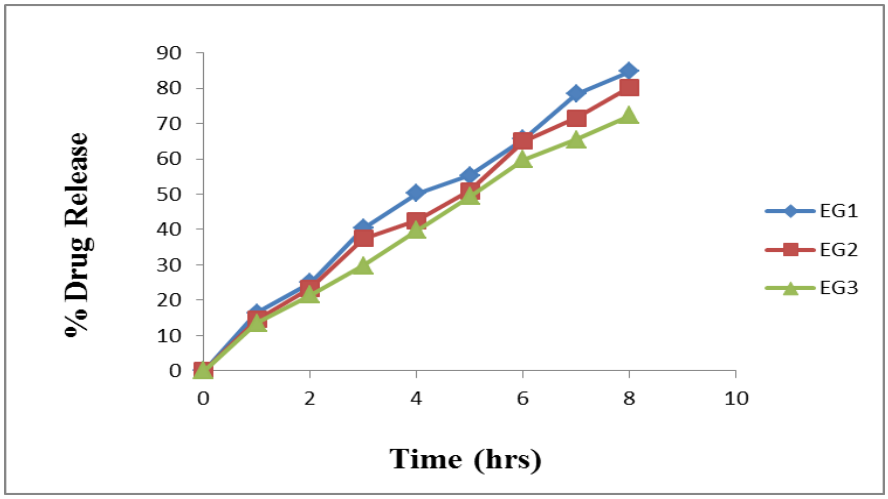

Fig. 12: Cumulative % drug release of Oxiconazole nitrate Ethosomal gels using carbopol 934.

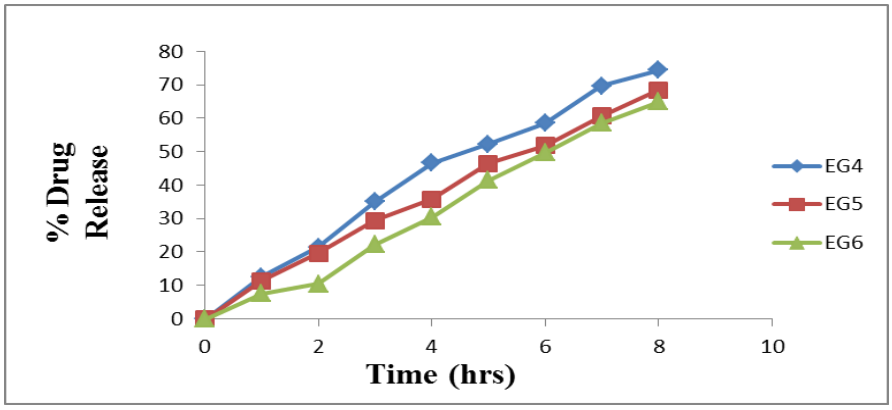

Fig. 13: Cumulative % release of Oxiconazole nitrate ethosomal gels using HPMCK4M.

In *in-vitro* drug release conducted for the optimized ethosomal formulation F14, the optimized ethosomal gel formulation EG1 and the marketed formulation Oxistat cream. The ethosomal formulation showed higher drug release than the gel and marketed formulation. The results are shown in figure 14.

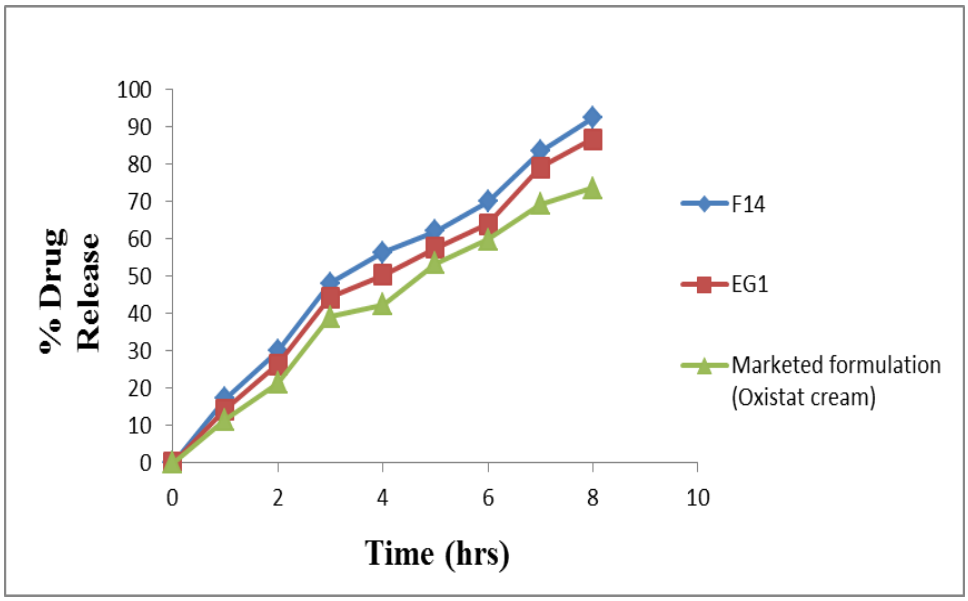

Fig. 14: Cumulative % drug release Vs time profile of optimized formulation (F14), optimized ethosomal gel EG1 and Marketed formulation (Oxistat).

Anti fungal studies

Anti fungal studies were performed and the selected formulations showed antifungal activity when tested by cup plate technique using Oxistat as standard solution. Anti -fungal activity studies were shown in Fig 15 and table 4. Formulation EG1 showed 24mm inhibition which was high when compared to the zone of inhibition of free Oxiconazole nitrate gel which showed 19 mm and marketed Oxistat cream showed 21mm.

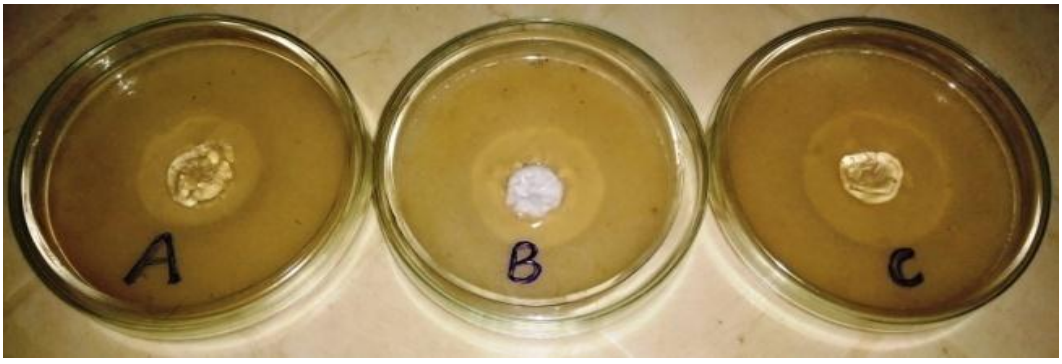

Fig. 15: Zone of inhibition of Oxiconazole nitrate Ethosomal gel EG1(C), Free Oxiconazole nitrate gel (A) compared with marketed Oxistat cream (B).

Table 4: Zone of inhibition of Oxiconazole nitrate ethosomal gel EG1, free Oxiconazole nitrate gel compared with marketed Oxistat cream.

Organism (Fungal strains)	Formulation	Zone of inhibition(mm)
Candida albicans	EG1	24 mm
Candida albicans	Plain Oxiconazole nitrate gel	19 mm
Candida albicans	Oxistat cream	21 mm

CONCLUSION

The results of present study clearly indicate that ethosomal gel formulations of Oxiconazole nitrate are possible. The incorporation of ethosomal systems in suitable vehicle such as gels represents an important step to get better skin-permeation and therapeutic results. The study confirmed that ethosomes were very promising carrier for the transdermal delivery of Oxiconazole nitrate which was revealed from the higher entrapment efficiency and better stability profile. Finally it was concluded that Oxiconazole nitrate loaded ethosomal gels were successfully formulated and offers advantages of rapid onset and maximum release of drug with reduction of side effects.

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CONFLICT OF INTEREST: Nil.

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