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Formulation and Evaluation of Posaconazole Drug Loaded Ethosomal Gel for Antifungal Activity

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ABSTRACT

The goal of this study is to create and test an ethosomal gel formulation of Posaconazole for antifungal activity. Topical drug administration is preferable for local action, and the efficiency of topically administered drugs is enhanced by liposomes, proliposomes, and ethosomes. The Classic method was used to create ethosomes from phospholipid, ethanol, polyethylene glycol, and purified water. Vesicle size, shape, optical microscopy, and entrapment efficiency were all measured in ethosomes. EP-3 outperforms the other formulation in terms of drug entrapment efficiency. Using carbopol 934 as a gelling agent, the best formulation (EP-3) was used to prepare the gel. The formulated gel formulation was tested for pH, viscosity, spreadability, and in-vitro release. The formulation EPG-2 has a better in-vitro drug release profile because it contains carbopol 980 at a concentration of 1% w/w. The current work also aims to improve the formulation's pharmaceutical acceptability.

INTRODUCTION:

In the past decades, topical delivery of drug by liposomal formulation have evoked considerable interest, it has been evident that traditional; liposomes are of little or no value as the carrier for transdermal delivery of drug, because they do not deeply penetrate the skin but remains confined to the upper layer of the stratum corneum. To overcome the problem of poor skin permeability Cave et al and Touitou et al recently introduce two new vesicular system transferosomes and ethosomes incorporated edge activator (surfactant) and penetration enhancer (alcohols and polyols) respectively to influence the properties of vesicles and stratum corneum1-3. Ethosomes are soft malleable vesicles composed mainly of phospholipid, ethanol (relatively high concentration), and water. These soft vesicles represent novel vesicular carriers for enhanced delivery to/through the skin. The size of ethosome vesicles can be modulated from tens of microns to nanometres. This carrier presents interesting features correlated with its ability to permeate intact through the human skin due to its high deformability4. The high concentration of ethanol makes the ethosome unique, as ethanol is known for, its disturbance of lipid bilayer organization; therefore when integrated into a vesicle membrane, it gives that vesicle the ability to penetrate the stratum corneum. Also because of their high ethanol concentration, the lipid membrane is packed less tightly than conventional vesicles, but has equivalent stability allowing a more malleable structure and improving drug distribution ability in stratum corneum lipid. As compared to classical liposomes that delivered drug to outer layers of skin, ethosomes were shown to enhance permeation through the stratum corneum barrier[5].

Fungal infections are one of the most concerning skin infections caused by various species of pathogenic fungi, affecting more than 25% of the world's population. Fungal infections can affect the skin, mucus membranes, nails, mouth, intestine, and vagina of a person. These infections can progress very rapidly especially in immunity compromised patients. Antifungal drugs are thus needed to be prescribed for the patients to treat the infections. Posaconazole is a potent triazole antifungal agent used in the prevention of invasive fungal infections due to aspergillosis and candida in high-risk patients. Posaconazole is a broad-spectrum, second-generation, triazole compound with antifungal activity. Posaconazole strongly inhibits 14-alpha demethylase, a cytochrome P450-dependent enzyme. Inhibition of 14-alpha-demethylase prevents the conversion of lanosterol to ergosterol, an important component of the fungal cell wall. Inhibition of ergosterol synthesis changes the fungal cell membrane composition and integrity, alters membrane permeability, and eventually leads to

fungal cell lysis. Compared to other azole antifungals, posaconazole is a significantly more potent inhibitor of sterol 14-alpha demethylase.

The present study aimed to statistically optimize the ethosomal gel for enhanced skin delivery of Posaconazole which was an effective candidate for the treatment of fungal infection.

MATERIALS AND METHODS:

Materials: Posaconazole was obtained as a gift sample from Yarrow Chem, Mumbai. Phospholipid was purchased from Himedia Laboratory, Mumbai. Ethanol, propylene glycol, and carbopol-934 were purchased from CDH chemical Pvt. Ltd. New Delhi. Double distilled water was prepared freshly and used whenever required. All other ingredients and chemicals used were of analytical grade.

Methods:

UV Spectral Analysis: The ultraviolet absorbance of Posaconazole was scanned from 200 to 400 nm then the wavelength of maximum absorbance was compared with reported λmax. Accurately weighed 10 mg of Posaconazole and dissolved in 100 ml of methanol in 100 ml of volumetric flask and prepared suitable dilution to make it to a concentration of 10 µg/ml and recorded the spectrum in U.V spectrophotometer (LAB INDIA UV 3000 +) in the range of 200-400 nm to find the λmax.

Infra-Red Spectral Analysis: The infra-Red Spectroscopy of the drug was carried out to ascertain the identity of drugs. A pellet of approximately 1 mm diameter of each drug was prepared by compressing 3-5 mg of drug with 100-150 mg of potassium bromide in KBr press. The pellet was mounted in 80 IR compartment and scanned between wave number 4000-600cm-1 using an FTIR – Brukers Alpha. (Pavia et al., 2001)

FORMULATION DEVELOPMENT AND EVALUATION OF ETHOSOME

Method of Preparation: The ethosomal formulation was prepared according to the method reported by Touitou et al. the ethosomal system prepared were composed of 1-3% phospholipid,10-30% ethanol, drug, 10% propylene glycol, and water to 100% w/w. phospholipid, drug and probe (Flurocein sodium) were dissolved in ethanol/propylene glycol mixture, the mixture was heated to 30 °C in a water bath. The double-distilled water heated to 30 C was added slowly in a fine stream, with constant mixing (mechanical stirrer) at 700 rpm in a closed vessel, mixing was continued for additional 5 min. The system was kept at 30ºC

throughout preparation. The final milky solution of twosomes was left to cool at room temperature.

The preparation was homogenized by using a vertex shaker for15 min. Liposomes were prepared by thin-film method by dissolving the phospholipids and cholesterol in minimum quantity of chloroform: methanol mixture (3:1 v/v) in around bottom flask. The organic solvent was removed in a rotary evaporator under reduced pressure to form a thin film on the wall of the flask. The final trace of solvent was removed under vacuum, overnight. The deposited lipid film was hydrated with an aqueous solution of the drug at 60rpm for one hour at room temperature. The preparation was vortexed using a vertex shaker for 15 min [8].

Table 1: Formulation of Ethosome

Formulation Code	Drug (mg)	Phospholipid (% w/w)	Ethanol (% w/w)	Cholesterol (mg)	Propylene glycol (% w/w)
EP-1	50	1	30	10	10
EP-2	50	1	30	10	10
EP-3	50	1	30	10	10
EP-4	50	2	20	20	10
EP-5	50	2	20	20	10
EP-6	50	2	20	20	10
EP-7	50	3	10	30	10
EP-8	50	3	10	30	10
EP-9	50	3	10	30	10

Visual examination: For visual examination ethosomal dispersion was first spread on the glass slide using a glass rod. The formation of multilamellar vesicles was confirmed by examining the ethosomal suspension under an optical microscope (Olympus) with a magnification of40x and 10x.

Transmission Electron Microscope (TEM) Analysis: Transmission Electron microscope (Morgagni 268 D) was used for this purpose. An electron microscope uses a beam of highly energetic electrons to examine objects on a very fine scale. Samples were prepared from dilution in distilled water followed by sonication and dropped onto a square of paper. After air drying, particles were coated with a negative staining material phosphor tungstic acid (PT) (to make the sample conductive) and covered with a grid. After a few minutes, the grid was

injected into the T.E.M. by grid injector and then examined by Transmission electron microscopy (9, 10).

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).

Zeta Potential Measurements: Zeta potential was determined using a zeta sizer. It indicates the degree of repulsion between adjacent, similarly charged particles in the dispersion system. The surface charge of twosomes is denoted as zeta potential, and it was determined by the electrophoretic mobility of ethosomes in U type tube at 250C, using a Zetasizer (Malvern, UK).

Drug Entrapment Efficiency: Entrapment efficiency Posaconazole ethosomal vesicles was determined by centrifugation. The vesicles were separated in a high-speed cooling centrifuge at 20,000rpm 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 a UV visible spectrophotometer at 427 nm [11]. From this, the entrapment efficiency was determined by the following equation,

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

Formulation of ethosomal gel: The ethosomal formulation (EP-3) showing higher entrapment efficiency and lower particle size is incorporated in a suitable gel base. The gel base is prepared using the following formula.

- Carbopol 980 NF 0.5- 2g
- Triehanolamine - 1.65ml
- Purified water - 100 ml

Carbopol 980 NF is weighed and dispersed in 100ml warm purified water by constant stirring. The triethanolamine is added and stirred until a viscous smooth gel is formed.

Preparation of ethosomal gel: The selected ethosomal formulation (ES) 30ml is centrifuged at 4oC,20,000rpm for 90 minutes to separate the ethosomal vesicles. The Ethosomal sediment which contains only the entrapped drug is collected, the entrapment efficiency is determined

in10ml. The ethosomal sediment is incorporated into 10g of gel base to obtain the ethosomal gel formulation, containing approximately 1% w/w of drug [12].

Appearance: The appearance was checked visually. After gelling, the clarity and color of the formulations were determined by visual examination of the formulations under light, alternatively against the 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 a 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.

Spreadability: The spreadability of gel was determined by modified wooden block and glass slide apparatus. A measured amount of gel was placed on a 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 Posaconazole 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 (area2.011cm2). This acted as a 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 a 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 analyzed for drug content using UV spectrophotometer (Lab India UV-3000+) at 427 nm against appropriate blank [13].

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 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 EPG-2, and free Posaconazole gel (prepared without ethosomes) were placed in the bores and the Petri-plates were incubated at 370C for 72 hrs in incubator. The zone of inhibition was observed and the radius of the zone of inhibition was calculated [14].

RESULTS AND DISCUSSION:

U.V. Spectroscopy of Drug: The maximum absorbance of Posaconazole reorder in Methanolic solution, which shows the high characteristics intense peak at 261 nm. The absorption spectrum of Posaconazole is very broad and the drug sample was almost 99% pure as analyzed by the official method is shown in **Figure 1**.

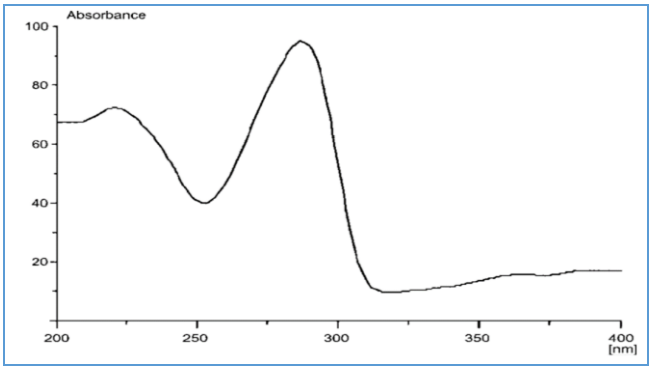

Figure 1: U.V. Spectroscopy of Posaconazole

FTIR Spectroscopy: The IR spectra of the drug were taken and compared with the drug and ethosome as expected, the IR spectra for formulation contained peaks corresponding to both the component drug and ethosome present as shown in **Figure 2**. The interpretation of I.R. spectra is listed in **Table 2.**

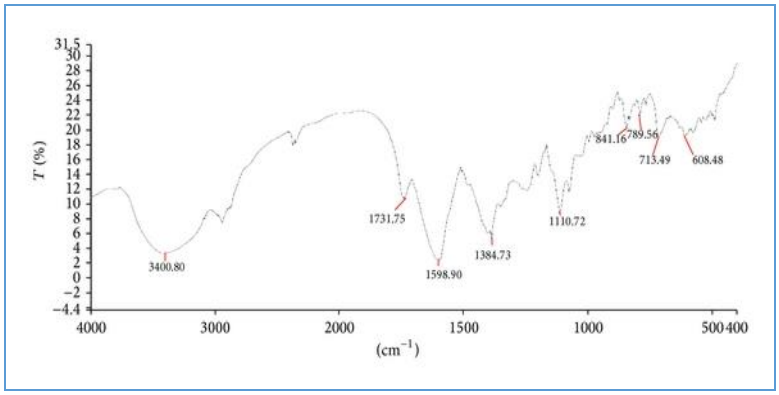

Figure 2: FTIR of Drug Posaconazole

Table 2: Interpretation of FTIR of Drug (Posaconazole)

S. No.	Frequency (cm^{-1})	Assignments
1	3400.80	-OH Stretching
2	1731.75	C-C binding with aromatic ring stretching
3	1598.90	COO^- stretching
4	1384.73	Ring vibration modes of C–O–C and C–O–H bonds
5	1110.72	C-C stretching

Formulation Characterization: The characterization of the formulation has been done by Visual examination, Zeta potential and particle size analysis, TEM analysis, drug entrapment efficiency.

Visual Examination: The visual examination of ethosomes was confirmed by using an optical microscope and the results are given below in **Figure 3**.

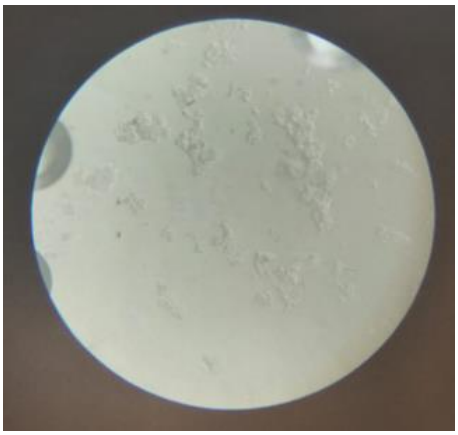

Figure 3: Microscopic image of Ethosomes

TEM Analysis: The SEM image indicated that ethosomes are found to be abundant, spherical in shape, and smooth. The TEM report is given in figure 4.

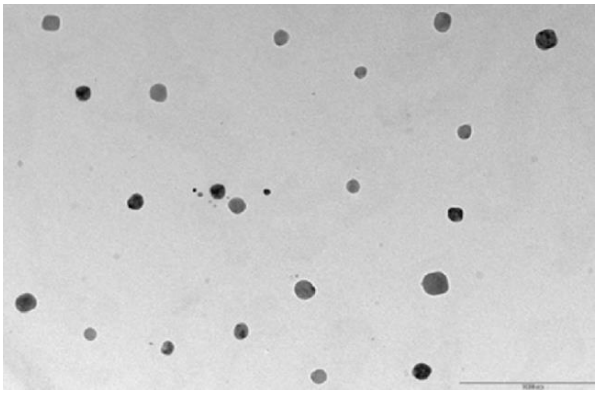

Figure 4: TEM images of ethosome formulation

Particle Size Distribution and Zeta Potential: The particle size and polydispersity index were found to be 19.9 respectively. The related report is given in **Figure 5**.

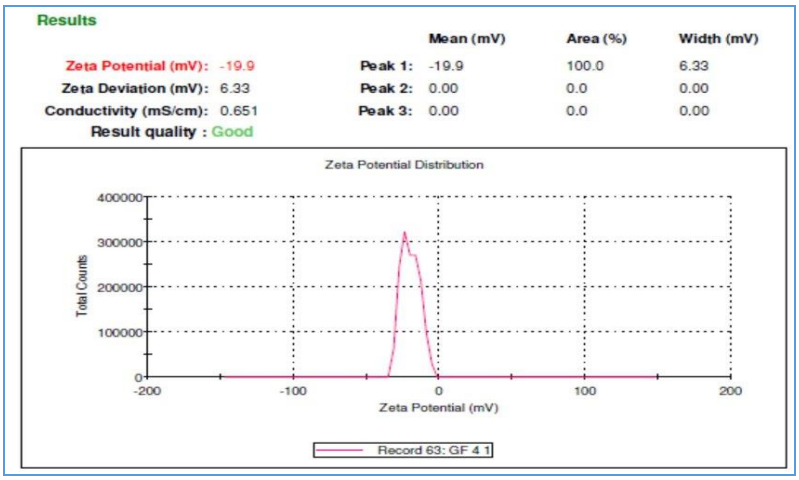

Figure 5: Zeta Potential of Ethosome formulation

Entrapment Efficiency: TMF7 demonstrated maximum entrapment efficiency among all the formulations compared to other formulations, as shown in **Figure 6**.

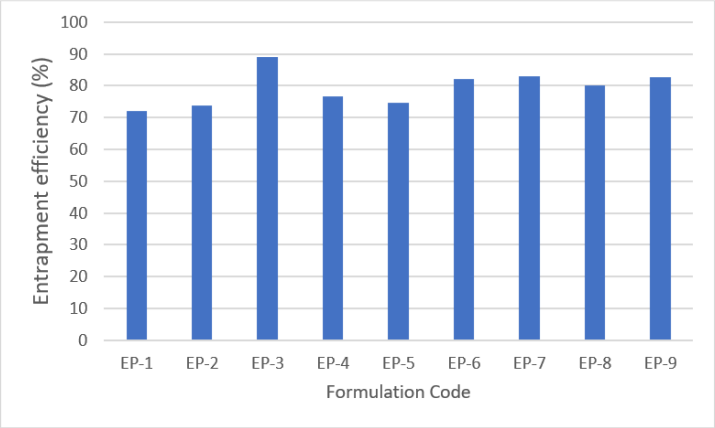

Table 6: Entrapment efficiency of Ethosome Formulations

Formulation of Posaconazole Loaded Ethosomal Gel: The gels were prepared by dispersion method using Glycerine, triethanolamine, and Carbopol 934 in different ratios.

Table 3: Formulation of Ethosomal Gel

Form. code	Ethosome The suspension (ml)	Carbopol 934 (gm)	Glycerine (ml)	Triethanolamine (ml)	Water (ml)
EPG-1	30	0.5	5	1.65	q.s
EPG-2	30	1.0	5	1.65	q.s
EPG-3	30	1.5	5	1.65	q.s
EPG-4	30	2.0	5	1.65	q.s

Characterization of Posaconazole Loaded Ethosomal Gel

Appearance: The appearance was checked visually. After gelling the clarity of the formulation color was determined by visual examination under light, alternatively against the white and black background. The color of ethosome is observed to be about pale yellow to colorless with a translucent appearance.

pH: In the range of 6.09 to 6.76, the pH of the formulations was considered sufficient to avoid the possibility of irritation. In **Table 7**, the result is shown. It was noticed that the optimized formulation EPG-2 pH was 6.45.

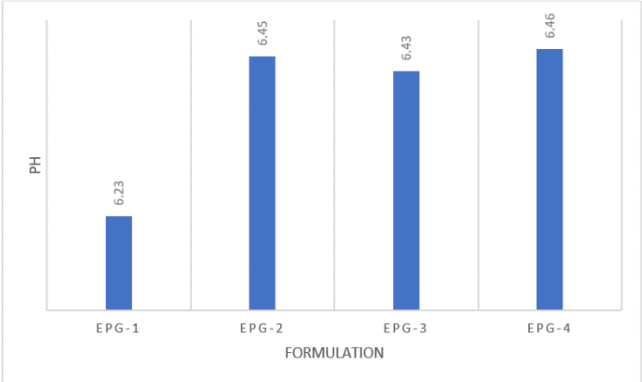

Table 7: pH of Ethosomal gel Formulations

Drug Content Analysis: The EPG2 formulation shows maximum % Drug content i.e., 94.76%, and thus selected as the final formulation. The results are shown in **Figure 8**.

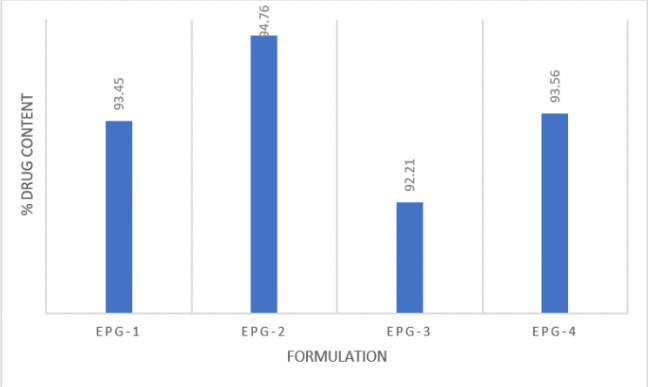

Figure 8: Drug Content Analysis of Ethosomal gel

Determination of Viscosity: The average formulation viscosity is in the 1221.479 to 3345.603 cps range. Table 9 shows the viscosities of all gel formulations and has been found to decrease by raising the shear rate, i.e. pseudoplastic behavior has been noted.

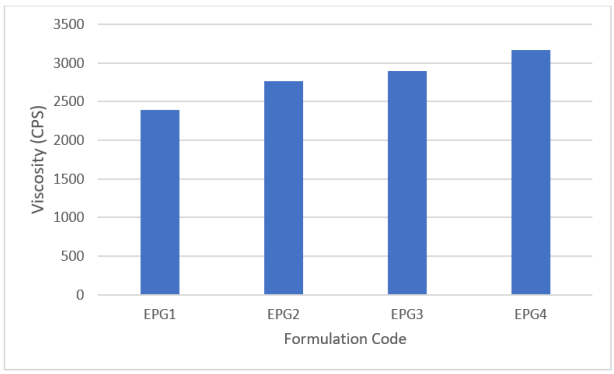

Figure 9: Viscosity of Ethosomal gel

Spreadability Study: Ethosomal gels agent exhibited spreadability values ranging from 10.14-13.56 g.cm/s.The spreading coefficient of various ethosomal gel formulations is given below in Figure 10.

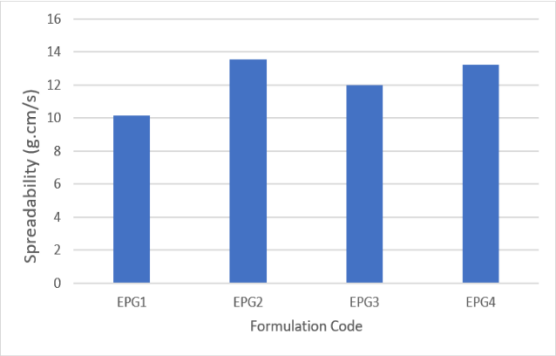

Figure 10: Spreadability Study of Ethosomal gels

***In-vitro* Drug Release:** The in vitro drug release studies were carried out across the cellophane membrane. The results of in-vitro release after incorporation of ethosomes in gels are shown in **Figure 11**. The cumulative percentage drug release for 12 hrs was highest for formulationEPG2 using carbopol 934.

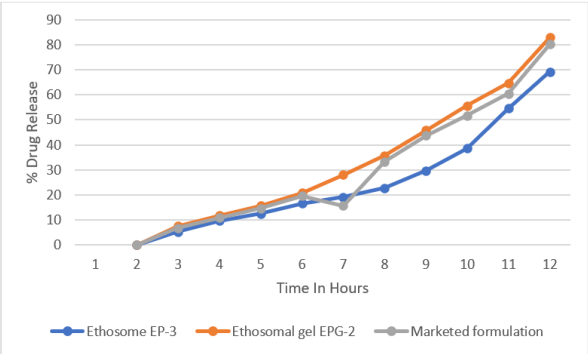

Figure 11: *In-Vitro d*rug Release

Anti-fungi studies: Anti-fungal studies were performed and the selected formulations showed antifungal activity when tested by cup plate technique using Oxistat as a standard solution. Antifungal activity studies. Formulation EP3 showed 26mminhibition which was high when compared to the zone of inhibition of free Posaconazole gel which showed 21 mm and marketed Oxistat cream showed 23mm.

CONCLUSION:

Incorporating ethosomal systems into appropriate vehicles, such as gels, is an important step toward improving skin permeation and therapeutic results. The study confirmed that ethosomes were a very promising carrier for the transdermal delivery of Posaconazole, as evidenced by higher entrapment efficiency and a better stability profile. Finally, it was determined that Posaconazole-loaded ethosomal gels were successfully formulated and offer the advantages of rapid onset and maximum drug release with reduced side effects.

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